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CONTAGIOUS BOVINE PLEURO-PNEUMONIA: CONTROL BY CULTURE VACCINES.

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I. INTRODUCTION.

THIS paper gives an account of researches that have been conducted in the Sudan during the past five years on the adaptation of living artificial cultures of pleuro-pneumonia virus for use as vaccines in the field.

It is not intended to enter into a preliminary discussion of the literature, since in consideration of the limited field covered by the present work it will be more convenient to refer to individual publications at the relevant points in the text. At this stage, however, it is acknowledged that considerable use has been made of the extensive bibliography compiled by Walker (1930).

Briefly stated, the position in regard to the active immunisation of cattle against contagious pleuro-pneumonia when the present series of researches was instituted was as follows :—

1.—" Natural lymph," either from a natural pulmonary infection or from an artificially produced subcutaneous lesion, has been extensively used as a vaccine, and, in fact, is still being used. This antigen, however, often caused the development of large swellings

at the site of injection, leading to extensive destruction of tissue and, in a considerable number of cases, the death of the vaccinated beast. By employing small doses and by introducing the lymph into selected sites, notably the tip of the tail, mortality as the direct result of vaccination was appreciably reduced ; similar improved results were obtained by dilution with glycerine and storage for varying periods before injection. No definite technique had been worked out and casualties still occurred on too large a scale, and further, when vaccinations were carried out without casualties little evidence of scientific value was provided regarding the subsequent immunity. The use of " natural lymph " is not, however, to receive attention in this paper and need not be further discussed.

2.—Artificial cultures of the virus in serumised Martin's broth had also been employed on a large scale, notably in recent years in East Africa. It had been recorded, partly in confirmation of earlier work, that while subcultures of a relatively small number of generations in Martin's broth were almost as dangerous as natural lymph, those of several generations were safe, and were efficient immunising agents. In principle it had been concluded that the best subculture to use was one of such age that it would produce universally " mild reactions " when injected, and that failing such a reaction in any beast there was no resultant immunity. In order to ensure, as far as possible, the development of reactions, the system of injecting two successive vaccines had to be employed, the second being either of greater virulence than the first, or, if of similar virulence, in a larger dose. In actual practice, moreover, it was generally found necessary to give more than two injections to some of the cattle in a herd, since in a number of instances no reaction followed the use of the first or second vaccine.

When the writer was first introduced to contagious bovine pleuropneumonia in the Sudan in 1925 vaccination on a small scale had already been started, the technique employed being that briefly outlined in the foregoing paragraph. On making a close study of the relevant literature, it was provisionally concluded that the recorded experimental evidence did not entirely support some of the arguments, and, in any case, information of much greater precision was necessary if the large scale use of culture vaccines was to be practised in the Sudan. The problems that presented themselves were largely interdependent, but may be approximately separated under the following headings :—

1. To find a substitute for Martin's broth.
2. To examine more fully the contention that a " reaction " is necessary if a vaccine is to produce immunity, and, arising out of this, the possibility of instituting a single dose vaccine.
3. To study more closely the effects of repeated subculturing of the virus in a standard culture medium, in particular with a view of establishing the significance of loss of virulence in regard to degradation of antigenic value.

4.—To study the effects on virus cultures of varying conditions and periods of storage.

5. Accumulation of evidence as to the duration of the immunity produced by a selected culture vaccine.

6. Arising out of the foregoing, the institution of some fairly definite policy for employing culture vaccines in the field in view of a more precise understanding of their properties and limitations.

It would be difficult to classify one's actual experimental records under the above headings, as the work has necessarily been otherwise divided. The general sequence will, however, be maintained.

II. Culture Media.*

The medium generally recommended for the maintenance of cultures of the pleuro-pneumonia virus has been Martin's broth with 10 per cent. of ox serum added. The preparation of this medium in the Veterinary Research Laboratory at Khartoum presented many difficulties. In the first place it entailed a good deal of trouble in the absence of sufficient apparatus and with the help of only partly trained native staff; in addition, there were very few pigs from which to obtain the necessary stomachs (since 1926 there have been no pigs). The possibility of manufacturing some other form of digest broth was considered, but as such a procedure would not eliminate all the local objections, and there seemed *a priori* to be no reason why ready-made commercial peptones should not be suitable, it was decided to test a few samples of these.

Experiments with Liquid Media.

In drawing up a scheme of experiments it had to be remembered that the pleuro-pneumonia virus was generally considered not to grow luxuriantly even in a suitable medium. In the past this conclusion might have been arrived at either on account of the rather inaccurate methods of standardising the reaction of the medium or—a point that appears to have received no attention—because it was not offered sufficient nutriment. These were, therefore, the main points investigated.

The " plain broth " or " plain peptone bouillon " of bacteriologists consists almost universally of watery extract of muscle to which 1 per cent. of commercial peptone and ½ per cent. of sodium chloride are added. In testing such a medium for pleuro-pneumonia cultivation samples were made consisting of watery heart muscle extract (500 gm. to 1,000 c.c.) to which ranges of peptones from ½ to 5 per cent. were added, all samples being provisionally adjusted to a reaction of *p*H 8·0 before final sterilisation. After sterilisation serum was added

* This work has already been briefly mentioned, *vide Ann. Rep. Vet. Dept. Sudan Govt.*, 1926, *Rep. of the Vet. Research Officer*; also Knowles, *Jl. Comp. Path. & Therap.*, 1927, Vol. XL, p. 230.

to all samples, ranging from nil to 40 per cent. Three parallel series were prepared, each being made with a separate brand of commercial peptone, and these series were again subdivided, one half being serumised with horse serum and the other with ox serum.

In carrying out the actual tests a standard seed dose of five drops of a stock culture in Martin's broth was added to 10 c.c. of medium. Readings were made daily for a week, the only feature assessed being the degree of opacity in the medium. It would be impossible to annotate degrees of opacity by the usual " plus " signs, but this difficulty was overcome by arranging the tubes in long racks, placing the most turbid tubes at one end and grading down to the least turbid at the other. Tubes were then noted as No. 1, 2, 3, . . . etc., in order of turbidity.

Owing to the large number of tubes of medium under observation this series of experiments was only repeated three times, but as the results were almost constant this was considered sufficient. The observations can be summarised as follows :—

1. The pleuro-pneumonia virus grows in " plain broth " provided it contains at least 1 per cent. of peptone. Growth, however, takes some days to become apparent and is at best scanty.

2. The addition of serum, whether of horse or ox, improves the growth, such improvement being marked as the proportion of serum rises from nil to 10 or 15 per cent. There is no significant further improvement when larger proportions of serum are incorporated ; in fact, by altering the optical qualities of the liquid the addition of serum beyond a proportion of 15 per cent. is a disadvantage.

3. There is no great difference between the growths obtained when horse or ox sera are added. In the actual experiments carried out, however, such little difference as could be noted was in favour of horse serum.

4. In serumised broth a progressively better growth is obtained as the proportion of peptone rises from $\frac{1}{2}$ to 2 per cent., but there is no significant further improvement when more peptone is incorporated. All three samples of commercial peptone were found to be suitable, but one gave consistently better results than the others, and is now the only one used (Difco Bactopeptone).

It may be objected that increased turbidity of the medium is not necessarily referable to a more luxuriant growth of an organism. With this objection in mind surface cultures on solid medium were attempted, using an agar medium prepared with what appeared after the first series of observations to be the best—serum broth, and it was found that when these were seeded with liquid virus cultures material from the more turbid broths gave rise to more surface colonies. The method of assessing these growths was not to count the number of organisms by the usual technique of serial dilution, but to allow one drop of liquid culture to spread over the surface of the agar medium and subsequently to arrange them " in order of merit " as was done with the broth cultures. This method

is possibly inaccurate, but as it was found to give fairly constant results it was accepted as satisfactory.

The reaction of the medium was next studied. The standard provisionally adopted in the foregoing experiments, namely *p*H 8·0, was selected on the recommendation of Dahmen (1923) and of Giese and Wedemann (1924). Reference to literature emanating from Kenya, where large scale vaccination with broth cultures had been practised for some years, failed to provide any information on the point; Walker (1920, 1921), although stating that Martin's broth was used, had made no note of its reaction. Later (1924) he stated that the medium "is now standardised by the *p*H method," again unfortunately omitting to record the actual reaction.

In any case, a reaction of *p*H 8·0 is a difficult one to maintain in practice and it was decided that as a new medium was likely to be adopted the whole matter might profitably be studied afresh.

Before proceeding to cultural experiments a large number of readings were made of the reaction of pleuro-pneumonia lymph, both from natural cases and from experimental subcutaneous lesions; the estimation of the reaction of the "tumour lymph" was in fact instituted as a routine post-mortem observation in all virus passage animals and controls in immunity experiments. In every case the reaction was slightly on the acid side of *p*H 7·4 (the normal reaction of the body fluids) and in consideration of this observation alone it seemed unlikely that a reaction of 8·0 should be the most suitable for artificial culture media.

Cultural experiments with serumised 2 per cent. peptone broth were carried out with samples ranging from *p*H 6·0 to *p*H 9·4. It was found that growth could be obtained in nearly every case between the extremes of 6·2 and 8·8, but the best growths were constantly confined to the range 7·2-7·6. Further, when tubes showing growth were stored on ice and tested at weekly intervals for viability, it was found that in the best range (7·2-7·6) successful transplants could be made up to a period of at least three months, while in tubes of higher acidity or alkalinity the period of viability was progressively less as the reaction differed from the optimum.

Arising out of the foregoing experiments a standard routine medium was adopted, consisting of ox heart muscle extract (500 gm.—1,000 c.c.) to which 2 per cent. of peptone and ½ per cent. of sodium chloride is added. Before final sterilisation in bulk the reaction is adjusted to *p*H 7·8, and it has been found that on distribution and sterilisation in tubes, bottles, etc., the reaction changes to about 7·4. In order to obtain a perfectly clear medium the bulk sterilisation is carried out at 120° C., and after distribution at 110°-115° C. Before use 10 per cent. of sterile horse serum is added and the lots of medium are tested for sterility by a few days' incubation. This simple medium, which has been in constant use since 1926, will permit of a visible growth being obtained within 24 hours and an apparently maximum growth in five or six days. In fact, it is not

only much more easily prepared than Martin's broth but is also a generally superior medium.

The possibility of adding sugars in order to improve the growth was considered. It was found that although the virus grew better in media containing fermentable sugars, notably glucose and maltose, the medium naturally became very acid ; on this account the use of sugars was abandoned (*see* also Dahmen, 1923).

Experiments with Solid Media.

Little need be recorded on the tests carried out with solid media. Preliminary trials with Martin's broth serum agar gave poor results, even when using Dahmen's (1923) technique of pouring serum over the finished medium instead of mixing before solidification. A series of experiments, parallel with those with liquid media, showed that the finally adopted broth solidified with agar was the most suitable medium. The technique of preparation that was found to be best was to add the serum to the sterile medium while still liquid and to mix thoroughly. In practice this medium has proved invaluable, being regularly used for checking the purity of cultures on transplantation ; it is also suitable for isolating fresh stains of virus from slightly contaminated material. In confirmation of Dahmen's observation, on Martin's broth agar it is to be noted that in order to obtain the most satisfactory results the agar medium should have a fairly dry surface at the time of inoculation. Before abandoning the discussion of culture media, it is worthy of note that very similar work was being done at about the same time by Nakamura, Futamura and Watamiki (1926) and that their observations led to similar conclusions. Since adopting the serum peptone medium in the Sudan experimental media have been prepared with ox, donkey, mule, goat and camel serum. In general there has been no significant difference in any of them ; horse serum, however, is usually obtainable and is the easiest to handle, and it has been retained throughout for routine use.

III. Method of Testing Immunity.

Before proceeding to a description of experiments in which the immunity of vaccinated cattle has had to be tested, it is necessary briefly to describe and assess the technique of such tests in the laboratory. The method used throughout has been to inject 1 c.c. of fresh pleuro-pneumonia lymph subcutaneously. This method is artificial, not only in the sense that infective material is introduced in an unnatural way, but also that the disease as it occurs in nature is not produced in non-immune animals. It is therefore necessary to consider the extent to which it is possible to correlate immunity as shown by resistance to such a test with immunity to natural infection. On this point the general evidence is favourable, and one specific instance described by Walker (1922) seems to place the correlation beyond doubt ; of 94 vaccinated and 34 non-vaccinated

cattle deliberately introduced into heavily infected herds and maintained there are over a year, 14 of the non-vaccinated and one only of the vaccinated beasts became infected with pleuro-pneumonia. Exact specifications of the vaccine employed and the interval between vaccination and exposure are not given.

Pure cultures of only one or two generations have never been employed as test viruses in the Sudan. It is generally stated, usually as the result of a small number of observations, that cultures of a few generations are as lethal as pure lymph. *A priori*, if cultivation in artificial media attenuates the virulence, it would be expected that the attenuation should commence—however slightly—from the first generation in culture. One small scale observation in the Sudan has certainly shown (as recorded later in this paper) that a second generation subculture may not kill more than half the non-immune cattle injected with it, and in an immunity test recorded by Walker and Kearney (1923) a first generation culture was used as the test virus and produced only a 75 per cent. mortality in the control cattle—eight out of twelve.

There is thus little doubt that if a sufficient number of cattle are observed the fresh lymph will be seen to produce a higher mortality rate than the youngest of pure cultures, and the latter will be objectionable as test material on account of the necessity for using relatively larger numbers of cattle in one's experiments, if for no other reason.

The following is an analysis of the results of inoculating 1 c.c. of pure fresh lymph in all but a negligible number of cases from an artificial subcutaneous lesion, into all control, virus passage, etc., beasts in the Khartoum Laboratory from late 1925 until mid 1931 :

A. Susceptible.	
1. " Typical " course of infection with progressive local swelling and high fever :—	
(*a*) Died or destroyed *in extremis*	120
(*b*) Recovered after infections of varying severity	9
2. " Arthritic " course of infection :—	
(*a*) Died	3
(*b*) Recovered after severe infection	1
B. Immune	3
Total	136

This record shows an over-all susceptibility rate of over 99 per cent., and an over-all mortality rate of 90·4 per cent. These figures should be recalled in considering the results of the immunity tests to be described later, since in these tests it has not been the practice to use more than two control cattle to any injection of virus, and in one or two instances an immune individual has been encountered. When this has happened no particular notice has therefore been taken of it.

A final point may receive mention before leaving the question of controls. Walker (1920) records that " exceptionally, unweaned calves were put in experiment : a synovitis frequently resulted and their use was discontinued for this reason." The actual number of calves used and the proportion developing synovitis is not recorded. In the above analysis of one's own controls only three sucking calves are included, all of which developed " typical " infections ; the four cases of arthritis and synovitis were all in adults, in one of which the etiology was confirmed by obtaining a pure culture of the pleuro-pneumonia virus. By pooling the two sets of observations therefore it is evident that this atypical form of infection may occasionally develop both in sucking calves and adults.

IV. The Necessity or Otherwise for a Vaccine to Produce a " Reaction " if it is to Produce Immunity.

If it were established that a living culture vaccine, in order to produce immunity must cause some kind of " reaction " when injected, the utilisation of such vaccines in the Sudan, entailing as they do the practising of multiple injections, would be confined to so limited a field as to render them of negligible account in the control of the disease. Reference to the technique of vaccination adopted in Kenya, which appeared to be the only country in which living culture vaccines had been generally used on a large scale, showed that at least two doses of vaccine were recommended, the first dose being injected into the tail and a second larger one into the sub-cutaneous tissue behind the shoulder. If, however, no " reaction " occurred to the dose injected into the tail this had to be repeated before proceeding to the inoculation of the second vaccine. Such a procedure would be quite impracticable throughout most of the Sudan, with its small veterinary staff, its large distances and indifferent communications, and the nomadic habits of most of its pastoral tribes.

Apart entirely from the practicability of this technique, there would remain the problem of the attitude to be adopted towards cattle in which one had failed by repeated injections to induce any reaction whatsoever. If it is admitted that some cattle may not react to the first dose it seems probable that still less of them will react to a repetition of it—assuming always that the culture itself is alive.

The foregoing two points have been discussed at some length by Hayes (1926) in Tanganyika Territory. There is no need to quote his remarks on the practicability of multiple vaccinations further than to note that they agree with one's own. In regard to the latter objection, however, he records that in one series of inoculations only 490 cattle of 4,974 could be induced to react. After demonstrating that this 490 could not possibly represent all the susceptible cattle in the herd he concludes that " in the eagerness of the Kabete Laboratory Authorities to achieve a perfectly safe vaccine they have so attenuated

the virus that it is in most cases unable to produce a reaction· *and hence presumably any immunity*,* when injected subcutaneously."

Finally, there appears to be little precise information as to what is the minimum result that may be accepted as a reaction. Obvious swelling at the site of injection would undoubtedly be accepted, but even if this occurs there can be no guarantee that it is not in part, or perhaps entirely, non-specific. It has to be admitted that when receiving one's first personal demonstrations of reactions one was in many instances in doubt as to whether they were not attributable rather to an abnormally developed tactile sense in the observer than to any response in the vaccinated animal. In any case, the necessity for a vaccine to produce a reaction was an accepted principle and the experimental evidence from which it had been deduced had to be closely studied before discarding it.

The desirability of a " reaction " appears to have been accepted at an early stage, but a search of the available literature has only revealed one reference supported by experimental evidence, namely, by Walker (1922), who carried out observations on 94 cattle that were vaccinated with virus cultures and were subsequently, together with unvaccinated controls, herded with infected cattle. When the experiment was completed the results were assessed as follows :—

1. Cattle which gave a definite reaction to inoculation resisted infection during the period they were exposed.

2. Forty-one per cent. of the non-inoculated cattle contracted pleuro-pneumonia.

3. Approximately 5·2 per cent. of the inoculated cattle which gave a doubtful or no apparent reaction to inoculation contracted pleuro-pneumonia.

The second of these conclusions may be left out of discussion as it concerns only the controls.

In considering the justification for the first and third conclusions, a study of the protocols shows that 94 cattle were vaccinated, of which 75 gave " reactions " and 19 did not ; when subsequently exposed to infection only one of the vaccinated cattle contracted pleuro-pneumonia and this was one of those which had not shown a reaction when vaccinated. Further study of the evidence fails to elicit details either of the age of the culture employed as a vaccine or of the interval allowed to elapse between vaccination and exposure to infection.

As against the necessity for a reaction, one may therefore argue that :—

1. The one beast which became infected is exceptional—one out of 94.

2. This beast is exceptional among the non-reactors—one out of 19.

3. There is no knowledge of the interval normally required for this particular vaccine to produce immunity ; further,

* Italics not in original.

all vaccinated cattle exposed to infection would not come into contact with the disease simultaneously, and it is conceivable therefore that one or more might do so before their immunity was strongly developed.

The records of this experiment leave no doubt that the vaccination was markedly successful ; the only point challenged is the necessity for a reaction after vaccination.

Earlier and later records published by the same worker do not support the contention ; in the earlier work the point is not raised, but in subsequent work it is assumed to be established. For example, working at an earlier date with cultures of his " Kajiado " strain, Walker (1920) records that " three cattle put in test with a 31 days old culture gave no apparent reaction but were found refractory when re-inoculated." Again, with his " N'gong " strain, " of three animals which did not react to the tail inoculation of a culture, one gave a slight reaction *when inoculated in the tail with virus of the same strain.** A doubtful reactor when re-inoculated with virus of the same strain did not react." The number of generations in subculture, and the interval between vaccination and testing are not stated ; these are not points at issue in this section, but that beasts which did not react on vaccination did not develop symptoms when tested with virus is evidence of their possessing a very efficient immunity.

In regard to later evidence, Walker and Kearney (1923) give an account of a very successful vaccination test in which 172 cattle were vaccinated with two doses of living cultures and subsequently tested. The data do not permit of analysis in great detail, but it may be quoted that there was an over-all reaction rate of 78·6 per cent. to the first injection, of 29·0 per cent. to the second, and of 18·9 per cent. to both. It is thus obvious that some cattle failed to react to either injection, but not one died in the immunity test.

A close study of the available literature has failed to reveal any other evidence in support of the necessity for a vaccine to produce reactions, and, apart from any question of their necessity, one's own observations on attenuated cultures suggest that the universal production of reactions would hardly be obtainable in practice, since progressive loss of virulence does not seem to be characterised by a lessening of symptoms in all cattle injected, but rather by the appearance in a lower proportion of individuals of symptoms of any kind.

In the earlier work carried out in the Sudan, commencing at the end of 1924 and reported by Knowles (1927), the East African technique was followed, and although it was found that cattle which had reacted to vaccination were immune when subsequently tested it was also noticed that some which had not reacted† were also immune. From that time, at first while assisting Knowles and later in continuing the work alone, it has been considered that a reaction following

* Italics in original.

† One's own inability to appreciate many so-called reactions when assisting Knowles may have had some influence in causing " non-reactors " to be so recorded.

vaccination is not a necessity ; in fact, on *a priori* grounds it is doubtful whether it is even desirable—at any rate if it is a true, specific reaction. It would seem that if immunity can be produced by vaccines of such attenuation that they produce no reaction, it is probably more risky in practice to use those which do produce one.

The point need not be further elaborated at this stage, since detailed records of experiments designed to provide other information will be seen amply to prove that a reaction is not necessary.

V. Single Dose Vaccines.

It has already been explained that a technique of vaccination entailing multiple injections would be largely impracticable in the Sudan. This fact was noted by Knowles (1927), and some experiments were actually carried out by him which showed that a single dose of living virus culture could produce strong immunity.

It is not to be inferred, however, that multiple injections of culture may not be advantageous ; the point is that in the Sudan they are largely impracticable. From one standpoint at least multiple injections may quite possibly be preferable, namely, in the sense that by giving them one will be indulging in a mild form of hyperimmunisation. In the laboratory also it seems that multiple injections may have played their part, not by showing themselves to be necessary, but by rendering inevitable such lapse of time between the injection of the first dose and the immunity test as would allow immunity to develop. Although it is essentially an active immunity that is aimed at, it is noteworthy that in the literature the interval required for it to develop has nowhere received attention, and in setting out records of immunity tests the interval has rarely even been noted.

The use of single-dose vaccines is so closely associated with the properties and limitations of attenuated living cultures of the virus that the investigation of the latter must first be considered. It may, however, be now stated that from 1926 inclusive all issues of living culture vaccine in the Sudan have been of the single-dose type and the experiments now to be recorded have been carried out with single doses only.

VI. Attenuation by Simple Subculturing.

The general principle that the virulence of the pleuro-pneumonia virus is degraded as the result of simple subculturing in an artificial medium has been long established, and on it has been based the work already discussed in connection with the use of virus cultures as vaccines. In the earlier work no attempt was made to estimate this attenuation with any degree of precision, and even more recently, although attenuated cultures have been extensively employed as vaccines, the possibility of some parallelism existing between loss of virulence and degradation of antigenic value has received insufficient attention. As noted by Walker (1930), " usually no distinction

is clearly made between the vitality and virulence of the virus." Such a distinction appeared to be of fundamental importance, since repeated subculturing might attenuate the virus in two ways, (i) so that it would produce no serious lesion, or perhaps no reaction of any kind, when injected, and (ii) so that it would produce no immunity. Further—a most important possibility—the two processes might not be coincident; having already concluded that a reaction was not essential to the production of immunity, it seemed that they were almost certainly not coincident.

Nothing could be found in the literature to give any information on this point. Walker (1920) concludes that first and second subcultures are as virulent as the virus from which they originated, that the twenty-fifth and subsequent subcultures produce less marked reactions, and that *the virulency of a culture remains constant for at least 17 generations after becoming fixed by passage in subculture*,* the lower standard of virulence being fixed at about the twenty-fifth generation. That he is still of that opinion is shown in his recent publication (1930), in which he says "The virus, although retaining its vitality, eventually becomes so attenuated in virulence *that it produces few if any reactions*,† and it is necessary to discontinue the use of the too attenuated pure culture vaccine and substitute a more virulent subculture . . ." This policy is, for practical purposes, undoubtedly sound, but it still appears that a "reaction" is considered necessary, and this conception continues to confuse the true issue. It is also difficult to admit the conception of a virus that will undergo progressive attenuation for 25 subcultures, will then remain "fixed" for a further 17 subcultures, and will thereafter continue to be attenuated.

In formulating experiments it had to be considered whether there was not some form of test that could be applied to a culture already so attenuated that it had gone beyond the stage of causing any reaction, in order to demonstrate not only the persistence of antigenic value but also the degree thereof.

Reference to Knowles' work indicated that the length of time required for immunity to develop might be a guide to the degree of attenuation. It will be noted in his paper (1. c, Table VI) that ten cattle injected with single doses of subcultures of generations ranging from a thirteenth to a sixty-sixth were all strongly immune when tested at an interval of 74 days. Further reference is made in his text to an earlier experiment carried out on the same lines, but which was discarded on the grounds that testing had been carried out with contaminated lymph (p. 234). This certainly was the case, but a more detailed study of the records which were retained in the laboratory showed that in spite of the confusing effects of bacterial contamination the animals appeared not to be immune to the pleuropneumonia virus. Further, the culture used for vaccination was

* Italics not in originals.

† Italics not in originals.

of the fifty-second generation, and the immunity test was carried out after an interval of 14 days. It thus appeared that there might be some relation between the number of generation of the culture and the interval necessary for it to produce immunity. If there were any foundation for this opinion it would seem from the two experiments just described that information of considerable value was contained in the observation that whereas a fifty-second generation culture would not produce immunity in a fortnight, a sixty-sixth generation culture would produce it in ten or eleven weeks.

Two experiments were therefore devised, the first to ascertain the interval necessary for a single dose of a culture of a " moderate " number of generations to produce immunity—using the two foregoing experiments as a rough guide—and the second, assuming some intelligible result to have been obtained in the first, using " very young," " young " and " old " cultures.

It is fortunate that before this was contemplated considerable regularity had been observed in the transplanting of stock cultures. This had always been done as nearly as possible at weekly intervals, so that in using the term " generation " it is implied that the number of generations represents the number of weeks in culture with weekly transplantations. It cannot be claimed that every transplantation was done at exactly seven days' interval, and as it is possible that this slight irregularity may have had some influence it must be mentioned. In any case, all cultures in stock were always transplanted simultaneously into a single lot of medium, so that whatever factors were at issue all strains were submitted to them equally.

Experiment of 14.11.26.

Eight bulls were vaccinated, each receiving 2 c.c. of a five days' growth of a forty-seventh generation subculture in serum peptone broth subcutaneously (behind the left shoulder). No " reaction " occurred in any beast. The intention was to test their immunity by injecting 1 c.c. of " tumour lymph " behind the right shoulder after intervals of two, four, six and eight weeks, or as near these intervals as the " tumour " in virus passage bulls would permit. The test dose of virus was to be injected on the opposite side of the body in order to eliminate any objection on the count of local tissue immunity. The programme was carried out with variations of only a few days from the standard adopted, and the results are set out in Table I.

In spite of the small number of cattle used, the results of this experiment are not difficult to assess. A single dose of a forty-seventh generation subculture did not produce appreciable immunity in a fortnight ; tests after four and six weeks showed that although immunity was developing it was not yet very strong ; probably after nine weeks it was solid. The results of the test were in any case sufficiently intelligible to warrant proceeding to the next one contemplated.

Experiment of 27.2.27.

Twenty-four bulls were divided into three groups of eight, the members of each group receiving subcutaneously a single dose of 2 c.c. of a

five days' growth of pleuro-pneumonia virus in serum peptone broth as follows :—

Group I.—Of a " very young," admittedly dangerous, culture—actually a second generation.

Group II.—Of " fairly young," probably safe, culture—actually a seventeenth generation.

Group III.—Of an " old " culture, the one selected being considerably older than that used in the preceding experiment—actually a hundred and fifteenth generation.

The further object, as in the preceding experiment, was to test two bulls of each group at successive intervals of two or three weeks (depending on the behaviour of the controls to each test, from which virus was to be obtained for the next test). As the direct result of vaccination four of the eight bulls in Group I died after showing the usual symptoms ; the remaining four survived after developing swellings of relatively small size at the site of injection, the largest being 5-in. × 3-in. on the fifteenth day. No beast in Group II or III showed any symptoms whatsoever.

Unfortunately, some of the cattle in this experiment died suddenly before their immunity could be tested (? forage poisoning). The series of tests was, however, carried out on the survivors, and is summarised in Table II.

The conclusions that can be drawn from the information in this Table are fairly definite ; a second generation subculture was dangerous, but produced a solid immunity in less than three weeks ; a seventeenth generation subculture was safe, and produced a very sound immunity in three weeks and an absolutely solid immunity in less than six weeks ; a hundred and fifteenth generation subculture although showing some degree of protective power, did not produce a serviceable immunity in nine weeks. From a purely immunological standpoint it would have been interesting to proceed further with cultures of the hundred and fifteenth generation in order to ascertain whether a strong immunity would be developed after a considerable length of time or whether its level would after no interval rise very high. From a practical standpoint, however, it was not worth while establishing the point, since one could immediately eliminate the possibility of ever using cultures of this age for vaccination in the field.

In summarising this section of the investigations it can be claimed that at least four principles are established, namely :—

1. Single dose vaccines consisting of living virus cultures are quite efficient.

2. Serum peptone broth permits a good growth of the virus, and is not markedly detrimental to its antigenic properties.

3. There is no necessity for a vaccine to produce a " reaction " in order to produce immunity.

4. The longer a virus has been maintained in artificial culture

TABLE I.

INTERVAL REQUIRED FOR IMMUNITY TO DEVELOP IN CATTLE VACCINATED SUBCUTANEOUSLY WITH A SINGLE DOSE OF 2 C.C. OF A FIVE DAYS' LIVING CULTURE OF PLEURO-PNEUMONIA VIRUS OF THE FORTY-SEVENTH GENERATION. DATE OF VACCINATION 14.11.26. NO REACTION IN ANY INDIVIDUAL.

Date of test.	*Interval since vaccination.*	*Bull No.*	*Symptoms.*	*Result.*
28.11.26	14 days	101	Progressive local swelling and high fever.	Slaughtered in *extremis* 15th day.
	14 days	102	" " " " " "	" " " 28th "
	Control	110	" " " " " "	" " " 28th "
13.12.26	29 days	103	Local swelling attaining maxim. of 12 × 8-in. on 15th day and disappearing 30th day. Mild fever.	Survived.
	29 days	104	Progressive local swelling and high fever.	Slaughtered *in extremis* 20th day.
	Control	112	Progressive local swelling, polyarthritis and high fever.	" " " 21st "
	Control	96	Slight local pain, no swelling, no fever.	Survived.
26.12.26	42 days	105	Local swelling attaining max. of 10 × 8-in. on 14th day and disappearing by 30th day. Mild fever.	Survived.
	42 days	106	Progressive local swelling and high fever.	Slaughtered *in extremis* 20th day.
	Control	114	" " " " " "	" " " 17th "
	Control	115	" " " " " "	" " " 26th "
16.1.27	63 days	107	Swelling never more than 1 × 1-in. No other symptoms.	Survived.
	63 days	108	Swelling never more than 1 × 1-in. No other symptoms.	Survived.
	Control	113	Progressive local swelling and high fever.	Slaughtered *in extremis* 17th day.
	Control	122	" " " " "	" " " 26th "

TABLE II.

INTERVAL REQUIRED FOR IMMUNITY TO DEVELOP IN CATTLE VACCINATED SUBCUTANEOUSLY WITH A SINGLE DOSE OF 2 C.C. OF A FIVE DAY'S LIVING CULTURE OF PLEURO-PNEUMONIA VIRUS OF THE SECOND, SEVENTEENTH, AND ONE HUNDRED AND FIFTEENTH GENERATIONS. DATE OF VACCINATION 27.2.27. NO REACTION IN ANY INDIVIDUAL.

Date of test.	*Interval since vaccination.*	*Culture generation.*	*Bull No.*	*Symptoms.*	*Result.*
20.3.27	3 weeks	2nd	128	No symptoms of any kind.	Survived.
		2nd	133	" " " " "	"
		17th	139	Local swelling attaining a maxim. of 8 × 6-in. on 12th day and disappearing by 21st day ? fever.	Survived.
		17th	140	No symptoms of any kind.	"
		115th	147	Local swelling attaining a maxim. of 6 × 4-in. on 20th day and persisting for over a month. Mild fever. Lost condition.	Survived
		115th	154	Progressive local swelling and high fever.	Died 26th day.
		Control	160	Progressive local swelling and high fever.	Slaughtered *in extremis* 19th day.
		Control	163	" " " " " "	Died 23rd day.

TABLE II (*continued*).

		2nd	135	No symptoms of any kind.	Survived.
		2nd	136	„ „ „ „ „ „	„
		17th	142	No symptoms of any kind.	Survived.
9.4.27	6 weeks	17th	143	„ „ „ „ „ „	„
		115th	157	Local swelling attaining a max. of 8 × 6-in. on 10th day, remaining thus until 17th day and resolving 48th day. Moderate fever. Lost condition markedly.	Survived.
		115th	159	Local swelling attaining a max. of 4 × 3-in. on 11th day and disappearing on 17th day. No fever.	Survived.
		Control	165	Progressive local swelling and high fever.	Died 21st day.
		Control	166*	No symptoms of any kind.	Survived.
		17th	141	No symptoms of any kind.	Survived.
		17th	144	„ „ „ „ „	„
30.4.27	9 weeks	17th	146	„ „ „ „ „	„
		115th	162	Progressive local swelling and high fever.	Died 28th day
		Control	168	Progressive local swelling and high fever.	Died 16th day.
		Control	170	„ „ „ „ „ „	Died 17th day.

* A naturally immune bull.

medium (at any rate in serum peptone broth) the longer it takes to produce immunity when injected subcutaneously.

By applying these principles to field practice one was enabled to produce vaccines of a type that could be used in the Sudan, namely, single dose vaccines of a " moderate " number of subcultures. It was also possible to give field veterinary officers some approximate information as to the length of time required for the vaccine to produce immunity. As a result, the issues of vaccine rose from 4,250 doses during the period October 1925-December 1926 to 17,590 in 1928 (the first full year after reporting the foregoing experiments).*

It is obvious that the general principle to be adopted in preparing vaccines for field use will be to employ cultures of the smallest number of generations that are safe. Observations on this practice have been recorded over a period of three years in collaboration with the field staff, and will be discussed in a later section.

VII. Effects of Storage.

The cultures employed as vaccines in the experiments recorded in the foregoing section were all " fresh " in the sense that they were injected immediately on withdrawal from the incubator at the period of approximately maximum growth—actually after five days' incubation at 37° C. The object of the experiments was, however, to apply their results to large scale field vaccination, and a too literal interpretation of observations made with " fresh " cultures was not to be accepted. At least four factors presented themselves, making it necessary to carry out observations on " stored " cultures :—

1. In order to supply a vaccine of approximately known qualities a fresh supply would have to be made for each requisition. This would entail a delay of at least five days.

2. The preparing of fresh vaccine for each requisition would often be inconvenient for the laboratory. (Nevertheless, until the experiments to be recorded in this section were completed the laboratory always did so).

3. In the case of the more distant stations even this procedure would be largely discounted because of the length of time necessary for the vaccine to reach its destination.

4. Veterinary officers in the field could not be certain that they would be justified in using vaccine that had been in their possession for even relatively short periods. In the absence of fairly precise knowledge of the rate of deterioration, to inject such vaccine might not only be a waste of energy but by doing no good might be harmful to the veterinary reputation.

In considering the " keeping qualities " of the culture vaccine

* A brief summary without discussion was given in *Ann. Rep. Vety., Sudan Govt.*, 1927.

attention had to be mainly focused on the antigenic value. As already noted by Walker (1930), no distinction is usually made in the literature between vitality and virulence ; the third factor, retention of antigenic value, is nowhere even mentioned. It was certain that this factor bore some relationship to both virulence and vitality, or in practice to the generation of subculture selected and the length of time it is stored. The question of " keeping qualities " was so urgently raised by field veterinary officers that some action had to be taken before it was possible to institute definitive laboratory experiments, and as the question was in any case of primarily field interest it was decided to carry out investigations under field conditions.

It was hoped that advantage could be taken of the steady eastward movement from Darfur Province of beef cattle which ultimately pass through Khartoum for export to Egypt. Arrangements were therefore made to supply the veterinary inspector in Darfur with a few strains of culture vaccine in the usual way for injection into these cattle. The scheme was to use some of the vaccine immediately on receipt and the remainder after intervals of one, two, three, etc., weeks, a few cattle of each batch being specially branded. The further intention was to pick out some of these cattle on their arrival at railhead (El Obeid) several weeks later, and send them to the laboratory for testing as to immunity.

This scheme unfortunately failed to provide very much information. On one occasion a consignment of vaccine was prepared for despatch by road, but after leaving the laboratory it was handed to the Governor of Darfur who was returning from Khartoum by air. The vaccine was used, and vaccinated cattle subsequently tested proved to be immune, but this successful issue had to be discounted because the vaccine had not been submitted to the usual conditions of transport by road.

On some occasions the specially marked cattle were never seen again, while on others, when the cattle were identified at El Obeid, the owners would neither allow them to be tested under guarantee of compensation nor sell them for any but ridiculously high prices.

The information obtained, although small in quantity, was of a definitely encouraging nature ; actually only two tests were carried out, the details being as follows :—

First Test. " Old vaccine," seventy-second generation in serum peptone broth. Issued 11.5.28, despatched (by air) 14.5.28, injected 19.5.28.

Second Test. " Fairly young vaccine," twenty-sixth generation in serum peptone broth. Issued 10.6.29, despatched 10.6.29, arrived 19.6.29, injected 23.6.29. Transport was by train for one day and by road for eight days (including some delay).

The immunity test, summarised in Table III, was carried out on 26.8.28, fourteen weeks after vaccination.

TABLE III.

Immunity Test of Four Cattle Vaccinated Fourteen Weeks Earlier with a Single Dose of 2 c.c. of a Seventy-second Generation Culture " Stored " for Eight Days after Issue.

Bull No.	*Symptoms.*	*Result.*
1	Fever 7th-17th days. Local swelling attaining a maximum of 10 × 8-in. on 10th day and resolving by 17th day.	Survived
2	No symptoms of any kind.	Survived
3	No symptoms of any kind.	Survived
4	No symptoms of any kind.	Survived
Control 244	Progressive local swelling and high fever.	Slaughtered *in extremis* 20th day.

The immunity test, summarised in Table IV, was carried out on 7.8.29, six and a half weeks after vaccination.

TABLE IV.

Immunity Test of Four Cattle Vaccinated Six and a Half Weeks Earlier with a Single Dose of 2 c.c. of a Twenty-sixth Generation Culture " Stored " for Fourteen Days after Issue.

Bull No.	*Symptoms.*	*Result.*
1	Swelling never more than 1 × 1in. No other symptoms.	Survived
2	As above	Survived
3	Progressive local swelling and high fever.	Died 16th day.
4	No symptoms of any kind for fifteen days.	Died 15th day of undiagnosed condition (P.M. impacted rumen).
Control 333	Progressive local swelling and high fever.	Died 15th day.
Control 334	As above.	Died 14th day.

In the first test a seventy-second generation subculture constituted the vaccine. From the observations recorded in the foregoing section it would not be expected that a " fresh " culture of this age would produce immunity in less than about three months, so that the recovery of all four bulls tested, after an interval of 14 weeks, three of them without showing any symptoms, showed that the vaccine had not appreciably deteriorated in a period of eight days. The conditions of transport were certainly good, but the cultures would have been submitted fairly continuously to a temperature of 30°-35° C.

In the second test a twenty-sixth generation subculture constituted the vaccine, which had been submitted to fairly severe conditions for 13 days ; in particular, it was almost certainly submitted daily to a temperature of 35° C. or more for several hours. From one's earlier experiments it would be judged that a " fresh " culture of the twenty-sixth generation would produce a solid immunity in from one to two months, and the immunity of three of the four bulls

tested after an interval of six and a half weeks indicated that if the vaccine had deteriorated it was only to a small degree.

Although the information obtained in these two tests was not extensive, it was at least encouraging and it justified the institution of a fairly large scale laboratory experiment.

From the immunological standpoint it seemed that observations should take the form of investigating attenuation, but in the sense that degradation of antigenic value and not of virulence would be the factor considered, since the work would be undertaken from the outset with cultures that would already have ceased to be virulent. Adopting this view, one would be investigating attenuation in artificial medium without subculturing as distinguished from that already investigated as the result of regular subculturing, and it seemed that it should be appreciable in the same way, namely, by a lengthening of the time required for the degraded cultures to produce immunity.

The viability of the pleuro-pneumonia virus in Martin's broth has been variably estimated, and experience in general has been that cultures live longer at relatively low temperatures. Generally speaking, the period of viability in favourable circumstances is considered to be at least two months. It has already been shown that in serum peptone broth the virus will remain alive for at least three months, and the greatest period has not yet been investigated. In studying the effects of storage it was, however, not considered necessary to continue beyond a period of three months, because in practice this length of time would almost certainly be the maximum required.

From the purely practical standpoint two sets of storage conditions had to be considered :—

1. Storage in the laboratory, where ice is available ; this being necessary in order to ascertain the degree to which one might carry a stock of vaccine, thus eliminating delay in complying with requisitions and inconvenience to oneself.

2. Storage under field conditions, implying throughout most of the year temperatures approaching and often exceeding 35° C.

The details of the scheme and observations were as follows :—

Experiment of 29.11.29.

A virus culture of the twenty-first generation was selected and a large number of tubes of serum peptone broth inoculated. After incubation for five days at 37° C., they were divided into two lots, one of which was further stored at a temperature of 37° C., and the other in a mixture of ice and water. In order to reproduce as far as possible the conditions of storage of vaccine for issue all tubes were sealed.

Every week from the second week onwards two or three tubes were taken from each lot, tested individually for vitality by making subcultures on solid and in liquid media, and the tubes of each lot mixed to obtain an average sample. Four bulls were then injected with a single dose of 2 c.c. of culture subcutaneously, namely two with each sample. This procedure

was continued until the twelfth week in the case of cultures stored in the ice chest, and the eleventh week in the case of those stored in the incubator (in the latter case the stock of cultures was exhausted by the eleventh week). The experiment was completed by subsequently testing the vaccinated cattle for immunity.

The most difficult problem that presented itself was to decide at exactly what interval after vaccination the immunity tests should be carried out. The results of experiments recorded in Section IV indicated that with a " fresh " culture of the twenty-first generation one would expect immunity to develop in some period under two months. In the present experiment it was fairly certain that some degree of further degradation would occur, and, moreover, this might not be of equal degree in cultures stored at freezing point and blood heat respectively. The two small tests on cattle vaccinated in Darfur had, however, indicated that after storage for a fortnight at a relatively high temperature the interval required for immunity to develop had not been sensibly increased.

Ultimately it was decided to carry out the immunity test in two halves :—

1. Cattle injected with vaccine stored up to a period of six weeks to have their immunity tested after an interval of a little more than two months.

2. The remainder to be tested later, the exact interval to be decided after studying the results of the first half of the test.

Actually the first group was tested three months after the last bulls in it were vaccinated, and the results were such that the second group was tested after twice this interval.

Unfortunately, at the time when the last vaccinations were being carried out cattle plague appeared in the laboratory and killed some of the cattle. Fortunately, on the other hand, in no case were both of a pair given identical vaccine infected.

In regard to the vitality of the cultures, it was noted that up to the sixth week there was no difference between the two lots, but from the seventh week onwards the samples stored in the incubator appeared to be losing vitality, taking two or three days to grow when transplanted ; from the eighth week a growth was not constantly obtained from every tube tested, and at the eleventh week no growth at all could be obtained. The ice chest samples gave good growths up to the tenth week, but at the eleventh week subcultures took three days and at the twelfth week ten days to show growth. There was no doubt as to the suitability of the culture media used for testing vitality, as tubes were taken from the routine lots in which stock cultures of several strains of pleuro-pneumonia virus were being transplanted weekly.

The results of the first half of the immunity test are shown in Table V.

It is not desirable to attempt to draw conclusions in any absolute

TABLE V.

IMMUNITY TEST OF CATTLE VACCINATED WITH PLEURO-PNEUMONIA VIRUS CULTURE OF THE TWENTY-FIRST GENERATION STORED FOR PERIODS OF FROM TWO TO SIX WEEKS AT 37° C. AND 0° C. RESPECTIVELY. VIRUS 1 C.C. INJECTED 7.4.30.

Period of Storage of vaccine.	*Temp. of storage.*	*Interval, vaccination to test.*	*Bull No.*	*Symptoms.*	*Result.*
2 weeks	37° C.	16 weeks	382	Progressive local swelling and high fever.	Died 27th day.
	37° C.		383	Swelling less than 1 × 1-in. No other symptoms.	Survived.
	0° C.		380	No symptoms of any kind.	,,
3 weeks	37° C.	15 weeks	377	Progressive local swelling and high fever.	Died 22nd day
	37° C.		389	No symptoms of any kind.	Survived
	0° C.		226	,, ,, ,, ,, ,,	,,
4 weeks	37° C.	14 weeks	390	Local swelling 9th-18th days maximum 4 × 3-in. No other symptoms.	Survived.
	37° C.		391	No symptoms of any kind.	,,
	0° C.		392	Progressive local swelling and high fever.	Died 22nd day.
	0° c.		393	No symptoms of any kind.	Survived.
5 weeks	37° C.	13 weeks	399	Local swelling 7th-14th days, maximum 2 × 1-in. No other symptoms.	Survived.
	37° C.		398	No symptoms of any kind.	,,
	0° C.		400	,, ,, ,, ,, ,,	,,
6 weeks	37° C.	12 weeks	402	Progressive local swelling and high fever.	Died 22nd day.
	37° C.		403	Progressive local swelling and high fever.	Died 30th day.
	0° C.		407	No symptoms of any kind.	Survived.
——		Control	495	Progressive local swelling and fever, increasing up to 12th day, on which day bull died of cattle plague.	
		Control	509	Progressive local swelling and high fever.	Died 16th day.

terms from the data in Table V. Restricting one's observations to relative terms, however, the deductions are as follows :—

1. The vaccine deteriorated on storage, both in the ice chest and the incubator, as shown by the incomplete immunity of the test cattle, although the test was carried out after a longer interval than the minimum calculated for fresh cultures of a similar generation to produce immunity.

2. In general, however, the deterioration was not very marked; of 16 cattle tested, eleven survived after showing mild or no symptoms.

3. Vaccine stored at 37° C. deteriorated more than that stored at 0° C. ; four out of ten bulls injected with the former succumbed when tested, while of six injected with the latter one only died.

4. It is impossible to offer any opinion as to the rate of deterioration in either case. In the first place the number of cattle employed was small, and the conditions of test were not identical ; for example, the cattle injected with vaccine stored over the longest periods were tested at the shortest intervals.

The results of this test were encouraging, and, although some deterioration had been recorded, there was no indication that all, or most of, the antigenic value had disappeared ; in fact, the main general conclusion was that the stored vaccines took longer to produce immunity than fresh ones. In arranging to test the remaining cattle it seemed that the main additional information obtainable would relate to the maximum period of storage. The interval between vaccination and testing had therefore to be sufficient to afford an opportunity for the most degraded antigens to produce an immunity. A further problem might also be solved, namely, as to whether such degraded antigens would produce a solid immunity after any period, or whether the immunity would be of low quality. The ideal interval of time could not be calculated, but the test was ultimately carried out after twice the interval allowed in the first half of the test, namely, about six months after vaccination of the last of the beasts to be tested. The results are summarised in Table VI.

It is here seen that 15 of the 16 cattle submitted to the test survived, and that, although not all immune to the highest degree, *i.e.*, to the degree of showing no symptoms of any kind, their immunity was nevertheless of a very high order, in that no beast was severely affected. The local swellings were rapidly resolved, and no systemic symptoms were shown. That one beast died may be attributed either to extreme degradation of the vaccine or to the fact that it was one of a pair tested at the shortest interval, or finally to individual idiosyncrasy ; probably all three played a part, but that the beast had some immunity is shown by its survival for 44 days, a period much greater than had ever been observed in any control or virus-passage beast during the whole period over which records have been kept in the laboratory.

An observation calling for mention is the apparently equal immunising value of cultures stored at 0° C. and at 37° C., as shown in this Table. It has to be remembered in assessing the data that no attempt was made to test the cattle at so short an interval as to show any difference if it were demonstrable. The deliberate intention was to allow a *long* interval, in order to permit of the most degraded vaccines proving their antigenic value if such were retained; the more rapid degradation at a high temperature was established in the first half of the test.

A most interesting observation is the survival of Bull 466, which had been vaccinated with a culture from which no subculture could be obtained on the day it was injected. It has not been possible to follow this observation up, but it is worthy of record, even if without comment.

Viewing the experiment as a whole, the conclusions may be stated as follows :—

1. The antigenic value of virus cultures is degraded on storage without transplantation, as in the case of maintenance with regular subculturing.

2. The degradation is appreciable in a similar manner, namely, from the longer period that the degraded virus needs in order to produce immunity.

3. The degradation is more rapid when the cultures are stored at relatively high temperatures.

4. Even by storage over a period that appears to represent the limit of vitality an appreciable antigenic value remains. (As noted in one individual case, it is not certain that antigenic value may not survive longer than vitality).

VIII. Duration of Immunity.

The duration of the immunity produced by the injection of living cultures of attenuated virus is, in the Sudan at any rate, of less importance than the time necessary for the development thereof, as will be shown in the section dealing with the practical application of one's experimental results. Before proceeding to these, however, the point requires attention.

In none of the available literature can any experimental evidence be found that would support any definite conclusion. It appears that there is a general opinion that vaccination immunity lasts about two years. The only statement of this nature that has been discovered is a quotation of Constant and Mesnard, ex Hutyra and Marek (1926) : " An eight-days old culture of the virus in Martin's bouillon serves as vaccine, of which cattle receive $\frac{1}{8}$ to $\frac{1}{2}$ c.c. subcutaneously, inoculated into the tip of the tail. The immunity thus produced lasts for at least two years, as immunised animals which have distinctly reacted to the inoculation withstand after two years a subcutaneous injection of 0·5 c.c. of pure culture without any reaction." In the absence of protocols and in consideration of the

TABLE VI.

IMMUNITY TEST OF CATTLE VACCINATED WITH PLEURO-PNEUMONIA VIRUS CULTURE OF THE TWENTY-FIRST GENERATION STORED FOR PERIODS OF FROM SEVEN TO TWELVE WEEKS AT 37° C., AND 0° C. RESPECTIVELY. VIRUS 1 C.C., INJECTED 30.8.30.

Period of storage of vaccine.	*Temp. of storage.*	*Interval vaccination to test.*	*Bull No.*	*Symptoms.*	*Result.*
7 weeks	37° C.	30 weeks	425	Swelling never more than 1 × 1-in. No fever, etc.	Survived.
	0° C.		428	Local swelling 14th-30th days, maximum 10 × 10-in. Little fever, never off feed.	,,
8 weeks	37° C.	29 weeks	440	No symptoms of any kind.	Survived.
	37° C.		441	Swelling never more than 1 × 1-in. No fever, etc.	,,
	0° C.		442	Local swelling 7th-21st. days, maximum 4 × 4-in. No fever, etc.	,,
	0° C.		438	Local swelling 7th-14th days, maximum 3 × 3-in. No fever, etc.	,,
9 weeks	37° C.	28 weeks	436	Local swelling 7th-17th days, maximum 3 × 3-in. No fever, etc.	Survived.
	0° C.		439	Swelling never more than 1 × 1-in. No fever, etc.	,,

TABLE VI. (*continued*).

	37° C.		448	Local swelling 5th-17th days, maximum 6 × 6-in. No fever, etc.	Survived
10 weeks	37° C.	27 weeks	453	Local swelling 7th-15th days, maximum 3 × 3-in. No fever, etc.	"
	0° C.		451	Local swelling 7th-12th days, maximum 4 × 4-in. No fever, etc.	"
	0° C.		452	No symptoms of any kind.	"
	37° C.		466	Local swelling 3rd-19th days, maximum 7 × 7-in. No fever, etc.	Survived.
11 weeks	0° C.	26 weeks	472	Local swelling 4th-16th days, maximum 7 × 7-in., mild fever 5th-10th days.	"
	0° C.		462	Progressive local swelling and high fever.	Died 44th day.
12 weeks	0° C.	25 weeks	464	Local swelling 15th-38th day, maximum 3 × 3-in. No fever, etc.	Survived.
——	——	Control	511	Progressive local swelling and high fever.	Died 24th day.
——	——	Control	515	Progressive local swelling and high fever.	Died 25th day.

evidence set out in the earlier sections of this paper, it is difficult to know the exact significance of this statement. It will therefore be of interest to record one's own few observations.

In a country such as the Sudan it is not easy to determine the duration of immunity, as the difficulties of identifying and acquiring vaccinated cattle after a lapse of months or years are almost insuperable. A few tests have, however, been carried out; the system of vaccination was not the same in every case, and in the earlier ones the age of the cultures employed as vaccine had not been definitely noted, but was approximately worked out from other records. In one case no controls were used. The details and the conditions of test are summarised as well as possible in Table VII.

The amount of evidence in this Table is very small, but it is at least consistent, and tends to show that immunity produced by either a fairly young culture or one of moderate age is of certainly more than two years' duration. An experiment is now in progress in which the local government dairy herd has been vaccinated with a single dose of a twentieth generation virus culture (all with absolutely no reaction). The intention is to test a few individuals of batches that are periodically cast from the herd. Five cows, Nos. 71, 86, 2, 47 and 91 in Table VII, have been tested after an interval of a little over a year in the case of Nos. 71 and 86 and a little over two years in the case of Nos. 2, 47 and 91, but it may be several years before any final conclusion is available.

The question of duration of immunity may therefore be left open for the present, but it may be certainly concluded that the period is so much greater than that required for immunity to develop as to constitute no objection on this count to the policy of vaccination.

IX. Practical Applications.

The conclusions arrived at in the earlier sections have been mainly expressed in relative terms, but in applying them to field practice it is necessary to translate them wherever possible into absolute terms, so that in undertaking vaccination on a large scale the properties and limitations of the vaccine can be appreciated with some degree of precision.

The conclusions have, briefly, been as follows :—

1. Serum peptone broth is as suitable a medium as serumised Martin's broth.

2. Subcultures of a few generations in serum peptone broth are dangerous, but the danger becomes reduced with progressive subculturing.

3. Cultures of the virus in serum peptone broth that have been subcultured for such a number of generations that no " reaction " follows their injection, are capable of producing immunity.

4. Solid immunity can be produced by a single injection of attenuated virus culture.

TABLE VII.

DURATION OF IMMUNITY TESTS ON VARIOUS CATTLE VACCINATED WITH LIVING CULTURES OF PLEURO-PNEUMONIA VIRUS.

Date of vaccination.	*Culture medium.*	*Generation.*	*Date of test.*	*Interval.**	*Beast No.*	*Symptoms.*	*Results.*
June, 1925 ($\frac{1}{2}$ c.c. tail) Oct., 1925 (1 c.c. neck)	Martin's broth	? 50th	13.3.27	18 months	C. 148	Local swelling, maximum 3 × 3-in. No other symptoms.	Survived.
					C. 149	Local swelling, maximum 5 × 6-in. No other symptoms.	"
June, 1925 ($\frac{1}{2}$ c.c. tail) Oct., 1925 (1 c.c. neck)	Martin's broth	? 50th	16.5.27	21 months	C. 151	Local swelling, maximum 4 × 3-in. No other symptoms.	Survived.
					C. 150	Local swelling, maximum 8 × 6-in. ? fever. Never off feed.	
				Control	176	Progressive local swelling and high fever	Died 19th day.
1.2.30 Single dose 2 c.c.	Serum peptone broth	20th	1.3.30	13 months	C. 71	Swelling never more than $\frac{1}{2}$ × $\frac{1}{2}$-in. No other symptoms.	Survived.
					C. 86	Swelling never more than 1 × 1-in. No other symptoms.	
				Control	607	Swelling, maximum 6 × 6-in., mild fever	"
				Control	647	Progressive local swelling and high fever.	Died 27th day
1.2.30 Single dose 2 c.c.	Serum peptone broth	20th	22.3.32	$25\frac{1}{2}$ months	C. 2	Swelling never more than 2 × 1-in. No other symptoms.	Survived.
					C. 47	No symptoms of any kind.	Survived.
					C. 91	" " " "	"
				Control	861	Progressive local swelling, etc.	Died 23rd day.

* From *last* injection.

5. The longer the virus has been maintained in serum peptone broth, the greater is the interval required for it to produce immunity.

6. Virus cultures destined for use as vaccine deteriorate on storage, the deterioration being shown by the fact that they require a longer interval to produce immunity ; the deterioration is more rapid at higher temperatures.

7. The immunity is of considerable duration—certainly two years and probably more.

The application of most of these is quite straightforward, the most generally useful being that there is no necessity for a vaccine to produce a reaction in order to produce immunity, and that immunity can be produced by a single injection. A further useful item of knowledge is that cultures of a relatively large number of generations (60 or 70) will produce immunity if one is prepared to wait the necessary length of time ; such culture could be used if safety is the essential consideration, or in more closely administered countries where there is a more adequate veterinary staff and quarantine rules can be enforced.

When evolving a field policy in the Sudan, it must be remembered that there is not at present, and there will not be for an indefinite period in the future, any question of eradicating pleuro-pneumonia. Although quarantine rules are adequate their enforcement in the case of nomadic pastoral tribes is almost impossible ; attempted quarantining may retard, but at the present stage of development will not prevent, the spread of the disease. The policy thus reduces itself to one of control, the procedure wherever possible being to slaughter visibly affected cattle in a herd and vaccinate the remainder. The slaughter of affected cattle is not always easy of accomplishment because in many instances it may be impolitic for a veterinary officer to make use of his full powers ; with the increasing confidence of the cattle-owning tribes this last difficulty is disappearing, but in any case the field policy cannot at present go beyond slaughter of affected animals and vaccination of the remainder.

The preparation of vaccine in the laboratory is relatively easy ; a simple medium is now available for use, and, with the knowledge that at low temperatures the deterioration of the vaccine is slow, it is possible to carry small stocks for short periods in the ice chest. With an approximate knowledge of the usual rates of issue it can be arranged that vaccine need rarely be kept in stock for more than a week.

Regarding storage of vaccine in provincial stations, the more rapid rate of deterioration at relatively high temperatures renders this in general inadvisable. It has to be remembered, however, that vaccination may be undertaken in two entirely different sets of circumstances, namely (i) in herds in which the disease has already appeared or which are immediately threatened, and (ii) in herds less immediately threatened and in which vaccination is undertaken as a

long-range precautionary measure. For the second of these the use of vaccine that has been in stock for some time is not open to great objection, since even after storage at 37° C. for two months or more it is capable of producing immunity, and in the circumstances a rapidly induced immunity is not of the first importance.

The duration of immunity may be dismissed with little discussion. It is certain that immunity lasts for many months, and it may possibly be found to last for some years. Knowledge up to this point is necessary in order to justify vaccination ; if the immunity did not persist for a considerably greater period than that required for it to develop, the benefits would not be proportional to the trouble entailed, at any rate, in the Sudan. No matter what the duration of immunity, however, it cannot benefit the young entry of the herd, so that in the absence of all hope of eradication wholesale vaccination is advisable at as short intervals as is practicable.

The most urgent matter to be decided, therefore, is the approximate number of generations of subculture to be used as vaccines, a consideration which resolves itself into determining a practical middle sector in the range of attenuation between the young but dangerous generations which produce a rapid immunity and the old but safe ones which produce immunity slowly ; in fact, it becomes necessary to determine as far as possible what are the youngest safe generations. On this point—probably the most desirable one on which to have fairly precise knowledge—observations in the laboratory have not entirely agreed with those in the field. It is therefore necessary to discuss both sets of observations at some length.

In regard to the laboratory observations, it has been provisionally assumed that, given an equal number of generations in subculture, all " strains " of virus culture will behave similarly. The justifications for this assumption are taken to be that no evidence has as yet appeared to suggest plurality, and that the culture medium has been as rigidly standardised as possible. In experiments designed to elicit information in relative terms there is little doubt that the assumption has been justified, since, whatever unappreciated factors may have been at work, all strains have been submitted to them equally. Since there must be a number of factors that would influence the rate of attenuation in culture, it seems that the only way to gauge the point at which pathogenic power is lost is, until these factors can be studied, to keep records of as many strains as possible maintained under what are hoped to be identical conditions.

It has already been recorded by Knowles (1927) that the cattle of the Sudan appear to tolerate younger generations of pleuropneumonia cultures than those of Kenya. His published records show that cultures of the seventh, eighth, and tenth generations were safe. Adding one's own observations to these, up to the time when it was concluded that laboratory tests as then conducted were a very uncertain guide (early 1930) records had accumulated of 29 cattle which had received vaccine comprising six strains of under ten

TABLE VIII.

NUMBER OF SWELLINGS AND DEATHS REPORTED FROM THE FIELD FOLLOWING THE USE OF LIVING PLEURO-PNEUMONIA VACCINE OF DIFFERENT AGES—ALL GIVEN SINGLE DOSE OF 2 C.C.

	Generation.									
Doses issued	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	Over 40	Total
	740	4,970	8,200	5,740	8,840	7,520	6,440	5,800	13,210	61,460
Abnormal swellings	51	120	??			1			20	192
Deaths	17	22	7			1			10	57

generations without deaths or serious swellings in any receiving vaccine for over four generations ; of generations between ten and 20, a total of 117 cattle had received cultures of nine strains without a single swelling having been observed. In 1929, when it was first decided to elicit similar information under field conditions, five strains were taken into use at various times, and, in order to have some kind of check, each one was before use as vaccine tested on two bulls at a 5 c.c. dose. The generations thus tested were the sixth, ninth, eleventh, fifteenth, and eighteenth, and in no case did more than a negligible swelling result. It must be admitted that experiments in the laboratory have been carried out under relatively ideal conditions, with careful disinfection of the sites of injections, using bacteriologically sterile syringes, and needles of fine calibre. Such conditions for the elimination of traumatism and secondary infection are not obtainable in the field. In the laboratory, therefore, subject to the reservation that unappreciated influences might be affecting the rate of attenuation, it was provisionally concluded that possibly after five or six, and almost certainly after ten, subculturings the virus became safe.

In the field, while the foregoing information was being collected in the laboratory, the evidence tended at first to confirm one's conclusions, so that, realising that the ultimate decision must come from the field, cultures of successively smaller numbers of generations were taken into use as vaccines. Veterinary inspectors were asked to report all considerable swellings and deaths, giving particulars of the issue of vaccine used. It is not imagined that all swellings have been reported, but it is highly probable that all deaths have been, and these, covering the three-year period 1928-1930, are summarised and roughly classified in Table VIII, no notice having been taken in compiling the Table of any possible secondary causation.

A gross assessment of this record shows that the over-all casualty rate has been small, deaths having been approximately 0·1 per cent. Limiting assessment to cultures of 15 generations and under, in which sector casualties consistently occurred, the death rate has been 0·33 per cent. It can thus easily be appreciated that, even if the pathogenicity of the virus cultures were the only factor at issue, it would be quite possible that any test on a laboratory scale would fail to anticipate such casualties.

In pursuing the examination further, it has to be considered whether the pathogenicity of the virus culture is the only factor at issue ; the question of secondary infections cannot be dismissed without discussion, more especially as the basis of the vaccine consists of a rich culture medium without any disinfectant preservative. Some of the reports that have been received from field officers show that septic infection has undoubtedly been responsible for some of the accidents, in the sense that local swellings have burst or have been surgically treated and found to contain true pus. In other instances, in spite of possible secondary infection, the swellings have

shown typical features of a "pleuro-pneumonia tumour." In such instances it is not certain that symbiosis has not been in some degree at issue.

In this connection it is interesting to record one laboratory examination of a bull which developed a large swelling following vaccination in the field. On arrival at the laboratory the swelling was seen to be open, and microscopic examination demonstrated the presence of many species of bacteria, mainly staphylococci. The beast was slaughtered and the swelling examined, when it was found that near the periphery it showed the characteristics of a typical pleuro-pneumonia lesion. From one area a few cubic centimetres of clear lymph were collected, and, as this appeared on microscopic examination to be free from bacteria, culture media were inoculated and two laboratory bulls were injected with 1 c.c. and ½ c.c. respectively of the lymph. Pure cultures of pleuro-pneumonia were obtained, but neither bull developed any symptoms. Three weeks later these bulls were tested with virus and both died.

Another observation worthy of mention is that the casualties that have occurred in the field (apart from those following the use of the youngest subcultures) have not in general been single and scattered but multiple and local. For example, the ten deaths and 20 swellings following the use of vaccine of over 40 generations in subculture all occurred in one series of 1,700 vaccinations using fifty-fifth and fifty-sixth generations. Over 6,000 doses of younger generations of the same strain had already been used in other districts without a single ill report. It is difficult to explain accidents of this nature without assuming secondary infection.

The determination of the youngest safe culture for field use is therefore not yet settled, in spite of observations recorded on over 60,000 vaccinations. A general assessment of the evidence, however, indicates that laboratory tests are liable to show that cultures are sufficiently attenuated while in large scale practice they seem not to be. Experience in the Sudan has shown that with cultures of up to 15 generations casualties occur with some regularity, and on this account, while awaiting an opportunity for studying the matter further, no cultures of less than 25 generations are being issued.

In deciding to discontinue the use of younger subcultures consideration must be given to the loss of efficacy entailed. This will take the form of a longer interval between vaccination and the production of immunity. From the experiments recorded in Section VI, and particularly those set out in Table I and II, it seems that if subcultures of the seventeenth generation produce an immunity (taking as one's standard the severe laboratory test) in about three weeks, and those of the forty-seventh generation in eight or nine weeks, those of the twenty-fifth or thirtieth should produce it in perhaps five or six weeks. These figures may be only approximate but are calculated to show that by using cultures of older generations the period of risk to the clean cattle in an infected herd will be

increased. It is impossible to forecast with any precision what loss the increased risk will entail, but the very open conditions of herding in the Central Sudan, in which area nearly all vaccinations are carried out, and general experience suggest that it will be small. It is, however, a necessary corollary to the policy of " safety first," which is being adopted until further field experience or laboratory research leads to the development of a more perfect technique.

X. Summary.

The work recorded in this paper has been carried out with the dual object of adapting cultures of the pleuro-pneumonia virus for use as vaccines in the Sudan, and of ascertaining with more precision than hitherto some details of their properties and limitations. The conclusions are :—

1. A suitable culture medium is a broth made with heart muscle extract with 2 per cent. of peptone and ½ per cent. of sodium chloride, standardised to a reaction of *p*H 7·8 before final sterilisation. After sterilisation 10 per cent. of serum—preferably horse serum—is added. The final reaction is in the region of *p*H. 7·4. A suitable solid medium can be made from the above by the incorporation of agar.

2. The pleuro-pneumonia virus when subcultured in this broth loses its antigenic properties at a slower rate than its pathogenicity. Living cultures of a few generations are dangerous when injected subcutaneously but produce immunity rapidly, while cultures of more generations are safe but require a longer interval to produce immunity. An attempt is made to apply approximately absolute terms of generations and times to this principle. It is also shown that appreciable antigenic value remains even in subcultures of a relatively large number of generations.

3. There is no necessity for a living attenuated culture to produce a " reaction " in order to produce an immunity.

4. A single dose of living culture, even of many generations, will produce an immunity.

5. Virus cultures (vaccines) stored without transplantation require a longer period to produce immunity than fresh cultures. This form of deterioration progresses somewhat more rapidly at higher temperatures. No attempt is made to translate this principle into absolute terms, but it is shown that even after storage at blood heat for a period up to the limit of viability a culture will produce a sound immunity after a sufficient interval.

6. The immunity produced is of considerable duration. A few local tests have shown it to last at least two years and there is no suggestion that this period approaches the maximum.

7. Indications are given for the application of the foregoing principles to field vaccination in the Sudan, and preliminary records are given of over 60,000 vaccinations. In all cases

but one no difficulty has arisen, but the determination of the youngest safe generations in subculture, on which depends the most rapid production of immunity, has not yet been accomplished. Field experience, in part at any rate on account of secondary complications, has not agreed with laboratory work, but it is provisionally assumed as the result of field observations that the general use of subcultures of 15 or less generations is attended by so much more risk than when older generations are used that those of appreciably greater age are indicated.

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CONTAGIOUS ABORTION OF SHEEP AND GOATS IN CYPRUS.

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Outbreaks of abortion in sheep and goats have been recorded in Cyprus for several years. The disease has been considered to have been the result mainly of debility due to adverse climatic conditions, accompanied by heavy parasitic invasion. Recently, however, in several outbreaks of the disease the high percentage of abortions and the good condition of the flocks involved has suggested a specific causal factor.[1] In one previously recorded outbreak a hundred abortions occurred in a flock of a thousand sheep.

From our existing knowledge of the disease it would appear that the principal causes of contagious abortion of sheep are a vibrio and paratyphoid bacteria. Caprine abortion has been less frequently recorded, except, of course, the abortion which is usually symptomatic of Malta Fever. Spirillary abortion was first noted in sheep in England by McFadyean and Stockman.[2] Paratyphoid abortion of sheep has been found chiefly in Germany and references to this type of abortion in English veterinary literature are few. Bosworth and Glover,[3] in 1925, drew attention to the disease affecting sheep in England, the causal organism being apparently one of the Salmonella group. Lovell[4] recently examined five strains supplied by these workers. He reported that all five strains appeared to be identical in morphology and antigenic structure. Only slight variations occurred in their carbohydrate reactions. A strain of German origin was also examined and appeared to be identical with those of British origin. The name given to this organism is *Bacterium abortus ovis*.

Bosworth and Glover[5] have recently reported that many outbreaks of this type of abortion have come under their observation during the last seven years, and that a considerable mortality may occur among the ewes.

An opportunity recently occurred for me to investigate an outbreak of this disease and from an expelled dead fœtus an organism was isolated which simulates *B. abortus ovis*.

The disease first came under my notice in January, 1931, when the Veterinary Inspector reported an outbreak of the disease implicating both sheep and goats. There were 640 sheep and 230 goats in the area. Fifty-four sheep and ten goats had aborted. At the termination of the outbreak the figures were as follows :—

Sheep in infected flocks	397
Number of abortions	113
Goats in infected flocks	174
Number of abortions	33

The flocks concerned appeared to be in fairly good condition, and very few symptoms of illness were observed before or after the abortion took place. The animals looked well but stopped grazing for about an hour before aborting. In this outbreak abortion took place when the fœtuses were three to four months old. The fœtuses were always dead when expelled. According to reports received from the flock owners, the health of the ewes and goats was apparently not impaired subsequent to the occurrence of abortion.

It was at first considered that the outbreak might be due to *B. melitensis*, although Malta Fever has not been confirmed serologically in indigenous animals. The disease has, however, recently been discovered in imported Maltese goats. Moreover, in a Mediterranean country one is naturally on the look out for this disease. Therefore, sera from a goat and a ewe which had recently aborted were obtained and tested against suspensions of *B. melitensis* and *B. abortus* Bang. Negative results were obtained.

We were then fortunate enough to obtain the fresh aborted fœtus from which the organism here described was isolated. Cultures were attempted from the stomach and heart blood of this fœtus and from the membranes on plain agar and in broth. Some of the tubes were incubated in an atmosphere of Co_2. A pure culture was obtained from the stomach, and the primary growth appeared to be more rapid in an atmosphere of Co_2. No growth was obtained in the cultures from the heart blood or the membranes.

Description of the Bacillus.

Morphology and Staining.

The organism isolated proved to be a short, stout Gram-negative rod with an occasional filamentous form present. The filamentous form disappears with repeated subculturing. The short rods are 1 micron to 2 microns in length. In older cultures the length appears to be slightly increased. It stains well with the simple laboratory stains, some of the bacilli appearing to be more intensely stained at their extremities.

Motility.—The organism is actively motile.

Cultural features.

Agar.—Growth on this media occurs rather slowly at 37° C. In 24 hours on agar slants the growth is not abundant. In fact, the growth is never heavy. It occurs as very small discrete smooth colonies which have a shining semi-transparent appearance. The growth is very similar to that of streptococci, and at this stage would appear to resemble that of *B. abortus ovis*. There is, however, no considerable increase in size of the colonies with further incubation.

Broth.—In 24 hours there is only a moderate degree of uniform turbidity. There is later a distinct tendency to sedimentation.

Litmus Milk.—There appears to be a very slight acid tinge in this medium in 10 to 14 days.

Potato.—Growth is poor and not characteristic.

Gelatine.—This medium is not liquefied.

Biochemical features.

Lead Acetate Agar.—No blackening develops along the line of inoculation.

Indol.—When testing with sulphuric acid and sodium nitrate, no indol or nitrates were detected in peptone water. When Ehrlichs reagent was used, however, a slight formation of indol was detected.

Carbohydrate Reactions.—Cultures were grown in peptone water plus 1 per cent. sugar, with litmus as the indicator. These were incubated at 37° for 14 days. Acid was produced from maltose, mannite, dulcite, glucose and lævulose. Saccharose, lactose and salicin were negative. We were unable to detect any gas formation with our sugars or with a batch supplied by Mr. Gosden, the Government Bacteriologist, but Mr. R. Lovell, who has kindly examined the organism, reports that he observed gas formation in the various media fermented. He also reports that it differs from the strains of *Bact. abortus ovis* isolated in England by failure to ferment xylose.

Resistance to Heat.—Broth tube cultures are not killed by an exposure for 20 minutes at 50° C., but are killed in 15 minutes at 58° C., that is, after 15 minutes at the latter temperature no subcultures were obtainable.

Pathogenicity for Laboratory Animals.

The pathogenicity of the organisms for small laboratory animals appears to be weak.

Guinea-pig.—One guinea-pig received 2 c.c. of broth culture subcutaneously, and one 2 c.c. intraperitoneally. There was no reaction in either case.

White Rat.—A rat inoculated with 1 c.c. broth culture subcutaneously remained normal. A rat received 1 c.c. intraperitoneally and died five days later. The organism however, was not recovered.

Pigeon.—A pigeon received 1 c.c. broth culture subcutaneously, and another 1 c.c. intraperitoneally. Both remained normal.

Rabbit.—One rabbit received 2 c.c. intraperitoneally. No reaction was exhibited.

The intravenous inoculation of 1 c.c. broth culture of an early generation proved fatal, the organism being recovered from the spleen, liver and heart blood.

A rabbit received 1 c.c. intravenously, and another rabbit 5 c.c. by the mouth, of a seventeenth generation broth culture. No reactions were shown.

Experiments on Sheep.

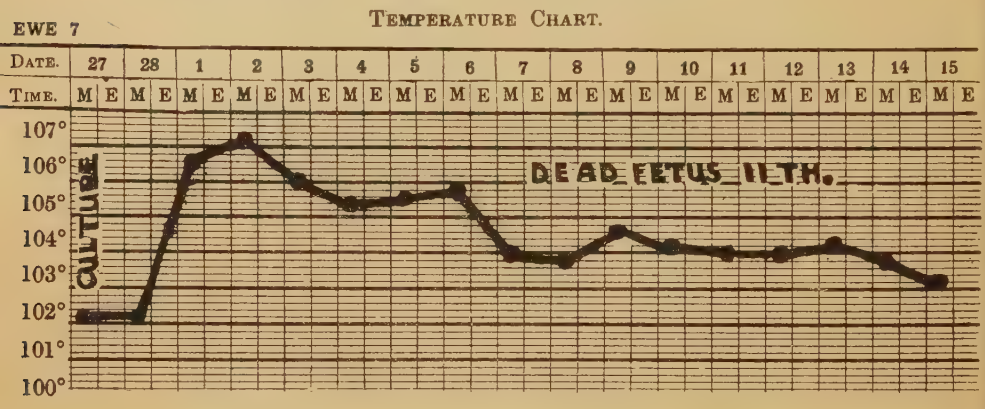
During the first experiments we had no opportunity of testing the organism on goats. Attempts were, however, made to produce abortion in ewes by the administration of cultures of the organism,

and in two instances by placing pregnant ewes in contact with artificially infected ewes.

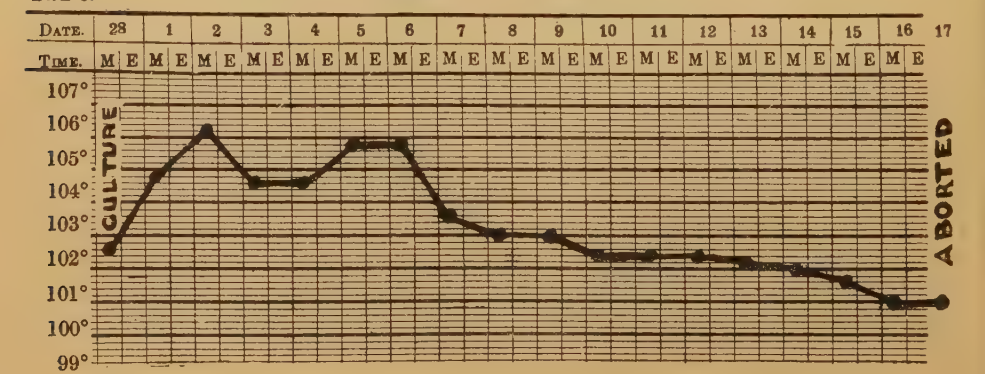
Artificial Infection.—Ewe No. 24, which was fairly heavy in lamb, received 20 c.c. of a broth culture by the mouth. Twenty-one days later she lambed, but the lamb died in 20 hours. The infecting organism was isolated from the spleen. The ewe had become extremely poor in condition and was killed *in extremis* two days after lambing. No definite temperature curve was exhibited.

Three ewes fairly well advanced in pregnancy were used for the next test, the inoculum consisting of a fifth generation 24 hours' broth culture.

Ewe No. 7 received 10 c.c. by the mouth and 2 c.c. *per vaginam*. A very marked temperature reaction occurred, reaching 107·2° F. on the third day. A dead fœtus was aborted 12 days subsequent to the inoculation. The organism was isolated from the heart blood, liver, spleen, bone marrow and stomach of the fœtus.


EWE 7
TEMPERATURE CHART.
DATE.
TIME.
27 28 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E
107°
106°
105°
104°
103°
102°
101°
100°
CULTURE
DEAD FŒTUS 11 T.H.

Ewe No. 8 received similar inoculations to No. 7. A marked temperature reaction occurred, and a dead fœtus was aborted 17 days subsequent to the inoculation. Cultures from the spleen of the fœtus and the fœtal sac were positive.


EWE 8.
TEMPERATURE CHART.
DATE.
TIME.
28 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E M E
107°
106°
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104°
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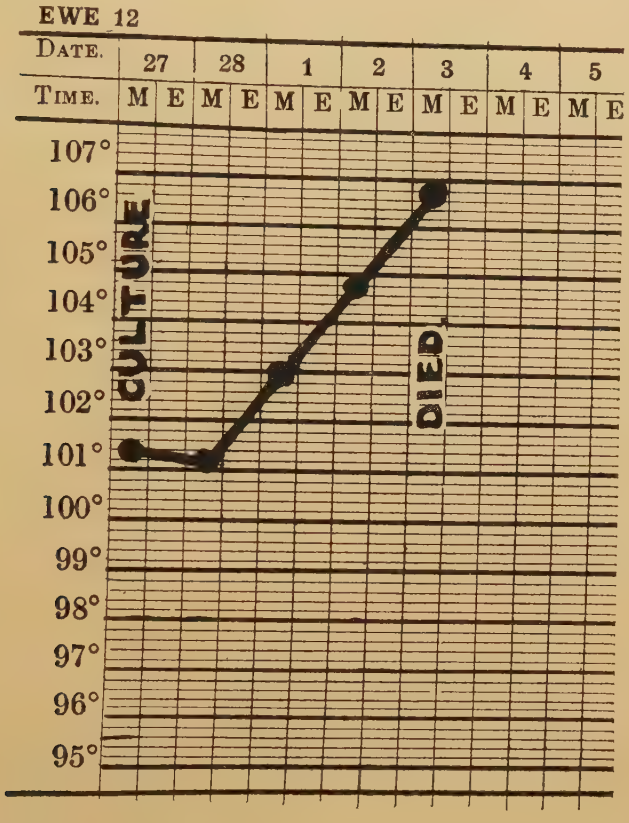
102°
101°
100°
99°
CULTURE
ABORTED

Subsequent to the inoculation both ewes, Nos. 7 and 8, fed poorly and lost condition. There was a slight brownish discharge from the vagina for several days following abortion. No other symptoms of disease were exhibited.

Ewe No. 12 received 5 c.c. of broth culture intravenously and 10 c.c. by the mouth. She died on the fourth day, after exhibiting a rapid rise in temperature. An acute enteritis was observed on post-mortem and the causal organism was isolated from the heart blood and the placental fluid.



Contact Experiment.—The previously mentioned three ewes, Nos. 7, 8 and 12, were kept in a small isolation pen, and, five days after No. 7 aborted and two days before No. 8 aborted, a pregnant ewe, No. 4, was placed in the pen with Nos. 7 and 8 to test the possibility of natural infection. No. 12 had died and had been removed from the pen 15 days before No. 4 was placed in the pen. The pen was not cleaned out during the course of the experiment in order to create the most favourable conditions for natural transmission.

Ewe No. 4 aborted a dead fœtus after being 19 days in contact with Nos. 7 and 8. Pure cultures of the organism were obtained

from the heart blood, liver, stomach and bone marrow of this fœtus. The ewe did not show any temperature curve. She subsequently developed a purulent metritis and died 12 days after aborting.

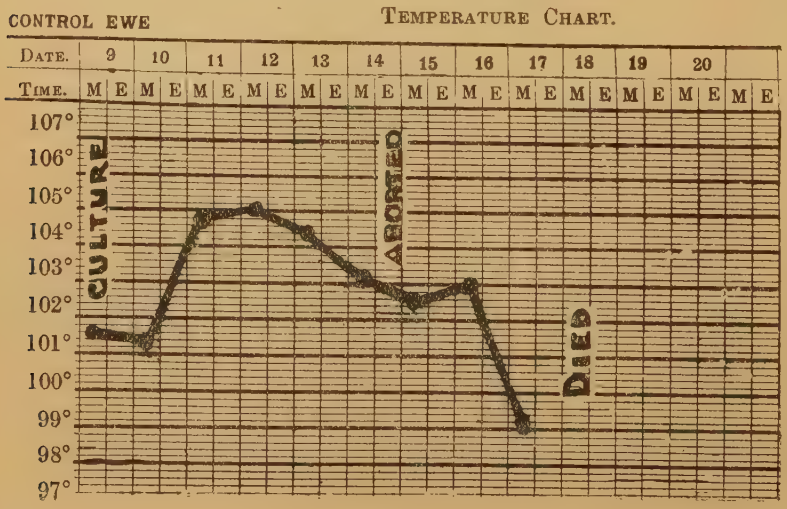
Pregnant ewe No. 25 was placed in contact with artificially infected ewe No. 24 (*vide supra*) on the day the latter was inoculated. She lambed normally 12 days later.

Vaccine Test.—One experiment was carried out to determine the immunising value of formalised culture. The vaccine consisted of a 48 hour broth culture which had been killed by the addition of 0·1 per cent. formalin.

Two pregnant ewes, Nos. 44 and 45, each received 5 c.c. and ten days later 10 c.c. of the vaccine subcutaneously.

In order to obtain a fresh culture for use as a test inoculation in this experiment, the strain was isolated afresh from a sheep. Sheep No. 17 received 30 c.c. of a broth culture intravenously. She died the following day. Post-mortem examination revealed severe hæmorrhagic inflammation of the abomasum and cæcum, and tumor splenis. A pure culture was obtained from the liver. A subculture from this was used as our test inoculum.

Nineteen days subsequent to receiving the second dose of vaccine, sheep Nos. 44 and 45 each received 20 c.c. of broth culture by the mouth. A control pregnant ewe, not previously vaccinated, also received 20 c.c. of the same culture by the mouth. She exhibited a marked temperature reaction, aborted a dead fœtus on the fifth day and died on the ninth day after inoculation. Post-mortem of the lamb revealed some blood-stained fluid in the thorax and pericardium and epicarditis. The ewe on post-mortem showed an acute metritis, congestion of the spleen, congestion of the mucous membrane of the abomasum, and inflammation of the small intestine. The organism could not be recovered from the organs of the fœtus or the ewe.



The two vaccinated ewes showed no temperature reaction to the virus inoculation and both subsequently lambed normally.

Some vaccinations were carried out in the infected area previously mentioned, but the disease apparently did not recur in this area during the following lambing season.

Susceptibility of Goats.—Goat No. 2 received 20 c.c. of broth culture *per os*. She aborted a dead fœtus 20 days subsequently. The organism was not isolated from the fœtus.

Goat No. 1 was placed in contact with the two vaccinated ewes, the control ewe and goat No. 2 on the day that the control ewe aborted. Goat No. 1 aborted a dead fœtus 20 days subsequently. The organism was not isolated from the fœtus.

Serological Reactions.

A few agglutination tests were carried out with inconclusive results. Sera from five sheep which had aborted in an infected flock and from ewe No. 7, an artificially infected ewe which had aborted five days previously, were tested against emulsions of the causal organism and *B. paratyphosus* B. The sera from one of the five and from No. 7 agglutinated both antigens in dilutions of 1 in 116 only.

The antigenic structure of the organism is being investigated by Mr. Lovell, at the Division of Bacteriology and Immunology of the London School of Hygiene and Tropical Medicine. He has informed me that the organism is apparently closely allied to the strains of *Bact. abortus ovis* isolated in England. It differs by its failure to ferment xylose, and by the fact that it appears somewhat deficient in type antigens.

Summary.

An outbreak of contagious abortion in sheep and goats in Cyprus is described.

The causal organism is apparently closely allied to the strains of *Bact. abortus ovis* isolated in England.

The association of goats with an outbreak of this nature is reported, possibly for the first time.

Abortion can easily be produced in sheep and goats by artificial infection with the organism.

In one small experiment two inoculations of formalised culture appeared to give complete protection against a subsequent dose of live culture, a similar dose of live culture producing abortion in a control ewe.

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"TICK-BORNE FEVER"

(A Hitherto Undescribed Disease of Sheep.)

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In the course of preliminary studies on the tick transmission of louping-ill, MacLeod (1932) showed that ticks collected in louping-ill districts were capable of setting up a febrile reaction in sheep. He also showed that animals which had recovered from this reaction were not immune to louping-ill.

In a previous paper (Gordon *et al.*, 1932) we described a field experiment in which 25 sheep immune to louping-ill were grazed with 24 non-immune "control" sheep on the tick-infested pasture of a louping-ill farm. While the sheep were on this pasture their temperatures were recorded daily. In the initial stages of louping-ill infection a temperature reaction occurs, and it was expected that such reaction would be observed in the "controls," while in the immune animals no rise in temperature would take place. As already recorded, however, in every sheep, both immune and control, a febrile reaction developed. This unexpected reaction in sheep believed to be immune to louping-ill has been the subject of further investigation which is here described, and which shows that the condition was a febrile tick-borne affection identical with that observed by MacLeod (1932). This condition we have, therefore, named "tick-borne fever." Detailed experiments recording the successful transmission of this disease by the nymphal and adult stages of the sheep tick, *Ixodes ricinus*, are described by MacLeod and Gordon (1932, a).

In this paper we record the result of our preliminary observations on the nature of the disease, and describe experiments to show that (1) the febrile reactions which occurred in louping-ill immune and louping-ill non-immune sheep while grazed on a "diseased farm" were due to "tick-borne fever" infection, (2) a specific immunity to this disease can be produced in sheep, and (3) louping-ill and "tick-borne fever" are immunologically distinct.

The Nature of the Disease.

The disease is characterised in its natural incidence by an incubation period of varying length, usually between four and eight days.

followed by a febrile phase which lasts about ten days, though it is often irregular and very prolonged. During the febrile period, the affected sheep appears dull and listless, and there is often a considerable loss of body weight. If the disease be uncomplicated by other infections recovery usually occurs, though a small percentage of affected animals die. In sheep killed during the febrile phase enlargement of the spleen has been the most constant macroscopic lesion found, and in a limited number of histological examinations of the spleen, mesenteric glands and central nervous system no definite pathological changes have been observed. The infection can be passed from sheep to sheep in series by the subcutaneous inoculation of blood from affected animals, and the incubation period following such inoculation is usually four days. The nature of the infective agent has not yet been established notwithstanding many attempts to demonstrate the presence of a bacterium, protozoon parasite, or filterable virus. In contradistinction to louping-ill, " tick-borne fever " cannot be induced in mice, and we have so far been unable to transmit the infection to any of the ordinary laboratory animals. The infective agent is always present in the blood of affected sheep during the period of fever, and for prolonged periods after the reaction has subsided. By experimental inoculation its presence has also been demonstrated in the mesenteric glands, spleen and central nervous system of affected animals. The difficulty which we have experienced in demonstrating the infective agent suggests that it may be similar in its nature to the cause of tick-bite fever in South Africa, which, after prolonged study, has been shown by Pijper and Dau (1930) to be due to a living virus of the *Rickettsia* class. The following experiments were carried out in four groups of sheep, each of which is described separately.

Group I.

The Field Reaction in the Louping-Ill Immune Sheep.

The sheep in Group I were the animals used for the serial passage of the febrile affection which occurred in the louping-ill immune sheep while exposed to natural infection. For the purpose of determining the nature of this fever, blood was obtained from one reactor (*vide* Gordon *et al.*, 1932, sheep serial No. 56) on April 15th, 1931, when its temperature was 107·8° F. To facilitate description, the blood thus obtained was named " immune reactor's blood " (I.R.B.). This blood was incapable of setting up louping-ill infection in sheep by intracerebral inoculation, and it was therefore assumed to be free from the louping-ill virus. A normal sheep was inoculated subcutaneously with 5·0 c.c. of this blood, and a temperature reaction commenced after an incubation of four days. Blood obtained from this animal during its reaction was inoculated subcutaneously into a second sheep which also reacted, and thereafter the infection

was passed in series through 26 animals. The temperature charts of five of these sheep are depicted in series in the left hand column of Chart 1, and the subsequent history of these sheep can be obtained by reading the charts from left to right. It will be observed that these five animals were tested with virulent " immune reactor's " blood at varying intervals after the primary reaction. As a control for the infectivity of each blood sample used for testing immunity, a normal sheep was also inoculated, and the fever curve exhibited by the control is depicted immediately above the chart of the tested sheep. For example, sheep No. 216 produced a typical primary fever curve after inoculation with I.R.B., and when tested with I.R.B. 30 days after recovery it was shown to have acquired a degree of immunity compared with its control depicted immediately above it. Twenty-nine days later it was again tested with I.R.B., and was practically immune. The record of the other four sheep shows that they were completely immune when first tested.

It may be concluded, therefore, that the reaction which occurred in the louping-ill immune sheep while exposed to natural infection was due to a specific infective agent which could be passed in series from sheep to sheep indefinitely. It may also be concluded that a specific immunity to this reaction can be produced in sheep.

Group II.

The Field Reaction of the Louping-Ill Non-Immune Sheep (" Controls ").

The Group II sheep were those used for the serial passage of the febrile affection which occurred in the louping-ill non-immune sheep while these were exposed to natural infection. As stated in a previous paper (Gordon *et al.*, 1932), it was believed that this febrile reaction was in some cases due to the louping-ill virus, and in others to this virus with, in addition, the infective agent responsible for the reaction in the louping-ill immune sheep. Blood, which we designated " control reactor's blood " (C.R.B.), was obtained from one of these reactors (*vide* Gordon *et al.*, 1932, sheep Serial No. 79) on April 15th, 1931, when its temperature was 107·4° F. This sample was proved to be free from louping-ill virus by intracerebral inoculation of sheep. By subcutaneous inoculation of 5·0 c.c. of this blood a reaction was produced in sheep, and passed in series through 27 animals. The charts of five of these are shown in series on the left of Chart 2. The subsequent history of each sheep can be obtained by reading the charts from left to right. It will be noted that all five animals were tested with virulent " control reactor's blood " at varying intervals after the primary reaction. It will also be observed that three were immune to the first test inoculation and the remaining two comparatively so, when compared with their controls.

It may be concluded from these experiments that, as in Group I, the infective agent responsible for the " reaction of controls " could be passed in series from sheep to sheep indefinitely, and immunity to this reaction could be produced.

Group III.

Animals used for Inoculation with Blood obtained from Sheep reacting to the Bite of Infective Ticks.

Blood of this nature was named " tick-borne fever blood " (T.B.F.B.), and was obtained from sheep reacting after infestation with ticks collected either from the pasture of the " diseased farm," or from infected sheep. Febrile reactions were regularly produced in normal sheep by subcutaneous inoculation with this type of blood, and these reactions were similar in nature to those produced in the sheep of Groups I and II. Five such reactions are depicted on the left of Chart 3, which also shows that in some cases sheep were immune after one inoculation, but in other cases more than one was required to produce immunity. Thus, as in the previous experiments, immunity can be demonstrated after recovery from the infection.

Group IV.

Sheep infested with Ticks collected either from the Sheep or from the Pasture of a Louping-Ill Farm.

The left hand column of temperature charts in Chart 4 are examples of the type of febrile reaction which was produced by infesting normal sheep with ticks collected from a louping-ill farm. This type of reaction was probably the one mistaken in some cases by Stockman (1918) for louping-ill, and shown by MacLeod (1932) to be immunologically distinct from that disease. Chart 4 also records the result of re-infesting some of these sheep with presumably infected ticks, and shows that they eventually became immune to tick bite, though several infestations were sometimes required. This is the febrile affection which we have named " tick-borne fever."

From these experiments it may, therefore, be concluded, as in the case of the reaction produced in Groups I, II and III, that a specific immunity to " tick-borne fever " caused by the bite of infective ticks can be produced in sheep.

Immunity Experiments between the Four Reactions.

In the foregoing experiments it has been shown that febrile reactions were produced in sheep with infective material obtained from four different sources. In the case of the reactions produced

in series in the animals of Groups I and II, the infective blood was obtained respectively from a louping-ill immune sheep and a louping-ill non-immune sheep which developed a febrile reaction while grazing on a louping-ill farm. The Group III reactions were produced with blood obtained from sheep which were reacting to the bite of ticks collected from the " diseased farm," and the Group IV reactions were " tick-borne fever," produced by infesting sheep with ticks collected from the same farm. The first two reactions were field reactions, the nature of which had to be determined, while the last two reactions were experimentally produced, and were believed to be " tick-borne fever." In order to determine that the two field reactions were the same, and that they were manifestations of " tick-borne fever " infection, immune sheep from each of the four groups were tested for immunity to the type of reaction produced in the three other groups.

Thus in Chart 1, No. 216, it is shown that a sheep which had produced a typical fever curve after inoculation with " immune reactor's blood " was comparatively immune when tested thirty days later, and almost completely immune when tested 29 days after the second reaction, while controls infected at the same time with the same material showed typical fever curves. The same sheep, when inoculated with " control reactor's blood " on two occasions, was completely immune. It was twice shown to be immune to " tick-borne fever blood," and when infested with known infective ticks was again immune. Chart 2, No. 343, shows that a sheep immune both to " control reactor's blood " and " immune reactor's blood " was immune to infection with known infective ticks. In Chart 3 there are several examples to show that sheep immune to " tick-borne fever blood " were immune both to " control reactor's blood " and to " immune reactor's blood," while Chart 4 shows that sheep which had recovered from the reaction caused by the bite of infective ticks were immune to inoculation with " tick-borne fever blood," as obtained from the sheep of Groups I, II and III.

As these experiments have been carried out a number of times, always with results as depicted in the Charts, it may be concluded that the field reactions which occurred in the louping-ill immune sheep while grazed on the " diseased farm " were due to " tick-borne fever " infection. In the case of the reaction in the louping-ill non-immune sheep it was probably due to this disease, with, in addition, louping-ill infection in some cases.

Immunological Difference between Louping-Ill and " Tick-Borne Fever."

Greig *et al.* (1931) and Gordon *et al.* (1932) showed that sheep which develop a febrile reaction after subcutaneous inoculation with louping-ill virus are immune on recovery to intracerebral infection

with this virus. Nos. 194 and 187, in Chart 1, and 185 in Chart 2 show primary curves after subcutaneous inoculation with louping-ill virus. After recovery these sheep were not immune to " tick-borne fever," and their final charts show that they were immune to intracerebral inoculation with louping-ill virus. Conversely, there are numerous examples throughout the four Charts which show that sheep immune to " tick-borne fever " were still susceptible to louping-ill infection. Since " tick-borne fever " can be induced in sheep immune to louping-ill, and louping-ill can be induced in sheep immune to " tick-borne fever," it may be stated that the two diseases are immunologically distinct.

Discussion.

MacLeod and Gordon (1932, b) have shown that the tick *Ixodes ricinus* L., is the vector of louping-ill virus. We now know that the same tick transmits the infective agent of " tick-borne fever," and in the course of other experiments we have encountered instances of both infective agents being harboured and transmitted by the same individuals. The close association of these two infective agents under natural circumstances may eventually prove to be of considerable importance in the etiology of louping-ill, especially in the production of those cases in which there is invasion of the central nervous system with virus. The only certain method of producing a typical clinical case of louping-ill by inoculation is by introducing the virus directly into the nervous system. If the virus is inoculated subcutaneously it is only in rare instances that it can pass the vasculo-meningeal barrier and be demonstrated in the central nervous system. The presence of the " tick-borne fever " infective agent in the brain of infected sheep suggests the interesting possibility that it may, in some way, favour the invasion of the central nervous system with the louping-ill virus. This aspect of the problem is at present under investigation.

Summary and Conclusions.

There occurs in the sheep on at least some of the tick-infested farms of Scotland a hitherto undescribed disease. When transmitted by inoculation it is characterised by an incubation period of about four days, followed by a febrile phase which lasts about ten days. The presence of the infective agent has been demonstrated in the blood, spleen and central nervous system. The mortality incidence is low, and after recovery most animals are comparatively immune to further infection. In animals killed during the febrile phase the only pathological change observed is splenic enlargement. The infective agent is transmitted by the tick *Ixodes ricinus* L. This condition we have, therefore, named " tick-borne fever."

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MacLeod, J. 1932. *Vet. J.*, lxxxviii, 276.
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" " " " " (1932, a.) *Parasitology* (in press).
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ABBREVIATIONS IN CHARTS.

I.R.B.	=	Louping-ill immune reactor's blood. That is, blood obtained from a louping-ill immune sheep which developed a febrile affection while grazing on a tick infested farm.
C.R.B.	=	Control reactor's blood. That is, blood obtained from a control sheep which had developed a febrile affection while grazing on a tick infested farm.
T.B.F.B.	=	Tick-borne fever blood. That is, blood obtained from a sheep reacting to tick bite.
Ticks	=	Infestation of sheep with ticks.
L.I.V.	=	Louping-ill virus.
+	=	Death.
---□---	=	The number in the squares between the temperature charts indicates the period in days which elapsed between the end of one reaction and the next inoculation.

CHART I.

°F.
108-
105-
102-
216
194
187
1
302
I.R.B.
L.I.V.
CONTROL.
C.R.B.
T.B.F.B.
T.I.C.K.S.
30
24
18
12
9
6
0
28
32

Chart II.

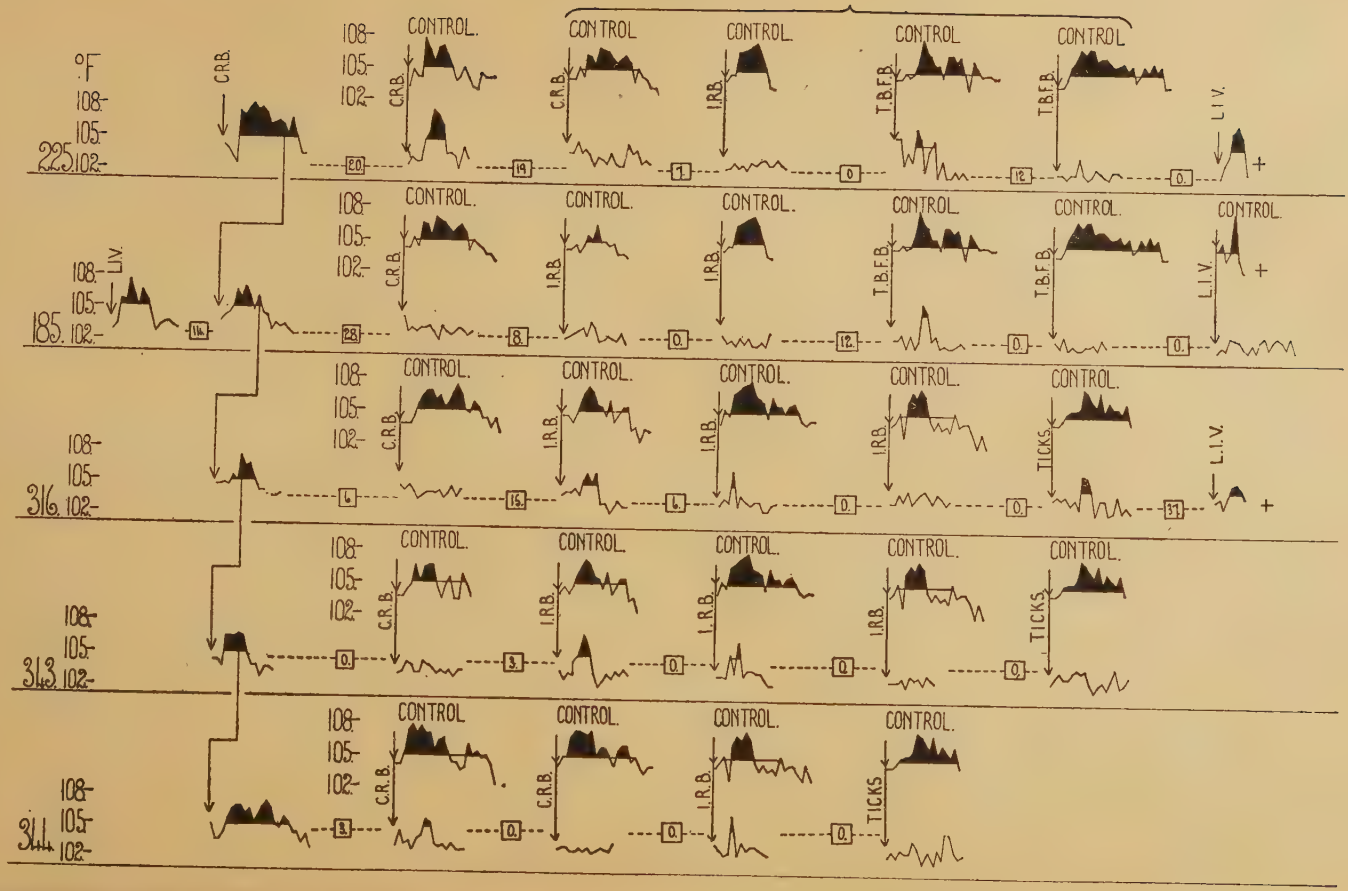
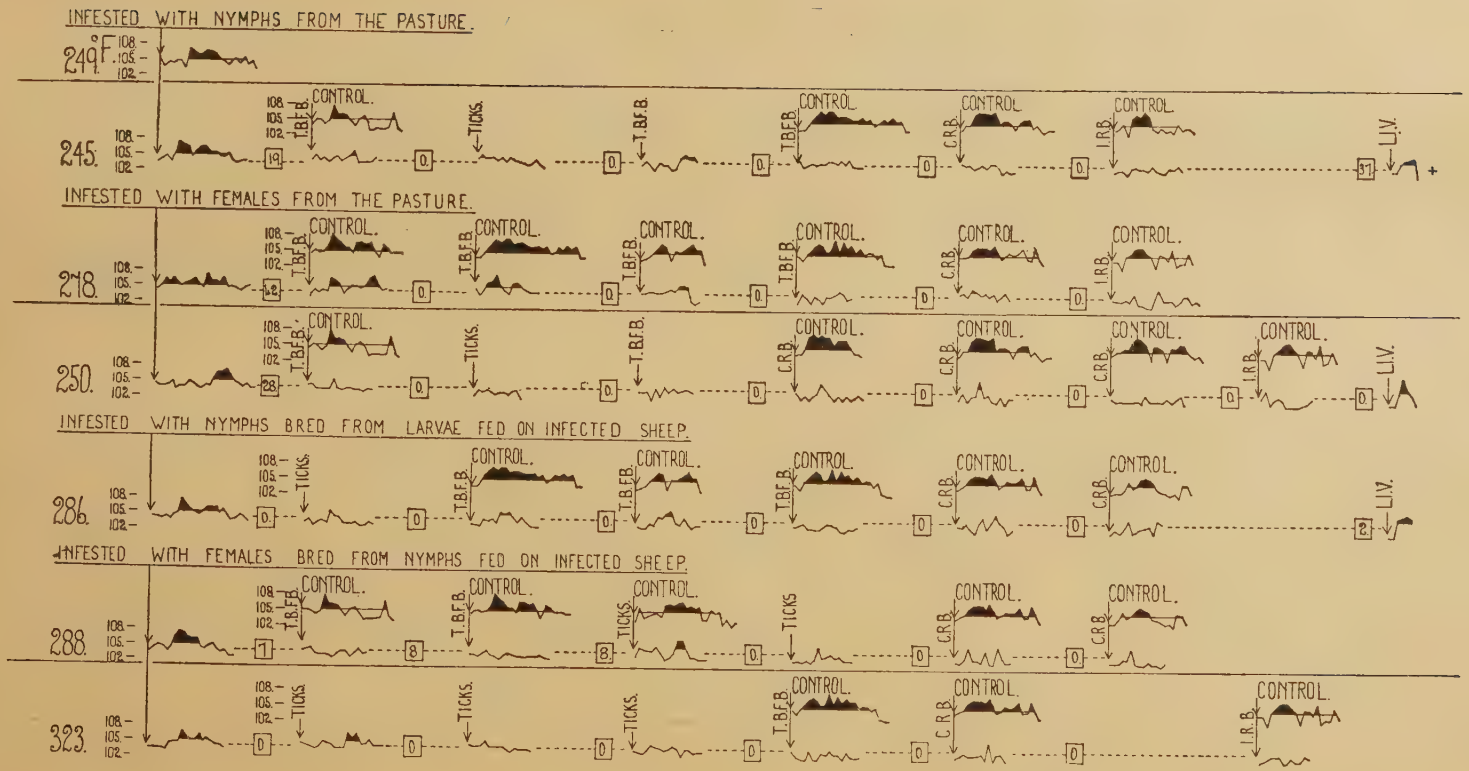

°F
108-
105-
102-
225.
185.
316.
343.
344.
C.R.B.
I.R.B.
T.B.F.B.
L.I.V.
TICKS.
CONTROL.
20
19
1
0
12
0.
14
28
8
0.
12
0.
0.
6
15
6
0
0
31
0
3
0
0
0
0
3
0
0
0

CHART III.

°F
108
105
102
296.
318.
300.
293.
314.
T.B.F.B.
CONTROL
C.R.B.
L.R.B.
L.I.V.
+
0
4
20
7
1
34
28
14

Chart IV.


INFESTED WITH NYMPHS FROM THE PASTURE.
249.
°F. 108. 105. 102.
245.
INFESTED WITH FEMALES FROM THE PASTURE.
278.
250.
INFESTED WITH NYMPHS BRED FROM LARVAE FED ON INFECTED SHEEP.
286.
INFESTED WITH FEMALES BRED FROM NYMPHS FED ON INFECTED SHEEP.
288.
323.
CONTROL.
T.B.F.B.
TICKS.
C.R.B.
I.R.B.
L.IV.

VACCINATION AGAINST BRAXY WITH FORMALINISED BUT LIVING WHOLE-CULTURES.

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Of all diseases of domestic animals braxy is by far the most important in Iceland. Before vaccination was introduced the losses were enormous, in many stocks up to 20 and even 50 per cent. of the total number of sheep. The introduction of vaccination in 1897 by C. O. Jensen and M. Einarsson was of great benefit to Icelandic farmers as the mortality sank soon afterwards very considerably and has never since reached its former height.

Several methods of vaccination have been tried. In 1916 Jensen gave a survey of the results of the different methods of vaccination employed here 1897-1906. From his paper we have taken the following table, summarising the results for the first ten years of experience in vaccination against braxy :—

Vaccine Used.	*Years.*	*Unvaccinated Controls.*	*Vaccinated.*	*Losses from Vaccination Per cent.*	*Losses from Braxy Per cent.*	*Total Losses Per cent.*
Dried Cultures	1897–1906	—	208,805	1·45	0·32	1·77
	1903–1906	13,576	—	—	4·22	4·22
Cultures and Serum	1902–1906	—	52,307	0·03	1·93	1·96
	1903–1906	20,872	—	—	5·04	5·04
Silk-threads with Imbibed Spores	1902–1906	—	15,934	0·03	4·65	4·68
	1903–1906	9,326	—	—	5·80	5·80
Total Controls		43,774	—	—	—	4·95
Total Vaccinated		—	277,046	1·05	0·97	2·02

From this table it may be seen that the efficiency of the vaccine employed is to a certain degree proportional to the losses from vaccination.

Since 1916 no reports have been published on the results of Jensen's vaccine.

In 1929 we introduced a new kind of vaccine after a series of preliminary experiments. The starting point for our experimentation was the work of *Ramon* on anatoxin, and MacEwen's experiments with formalin-killed whole-cultures as immunisatory agents against braxy. After some experiments with formalin-killed cultures we came to the result that the immunising stimulus was too slight and

too slow to be employed in practice and so we sought for a better method. This we found in employing the whole culture, not killed, but only formalinised so as to weaken the virulence of the culture but not kill it.

Since 1929 we have employed whole cultures, grown for 24 hours and then formalinised to 2·5 per cent. and left in the incubator for another 24 hours. The culture loses its toxicity rapidly after the addition of formaldehyde. One c.c. does no harm to a mouse (intravenously) after twenty-seventh formalinisation, although 1/20 c.c. is enough to kill it before formalinisation.

Preparation of Vaccine.

Beef- or horseflesh-bouillon (*p*H 7·5) with peptone (but no glucose) and minced meat is autoclaved in great flasks with a vaseline layer covering the surface. The contents of the flasks must be at 35-40° C. when they are inoculated with a vigorous (12-18 hour) culture of *Vibrion septique* and left at 38-40° for 24 hours. Then 2·5 c.c. formaldehyde is added per litre of culture and the whole left for another 24 hours. The culture is then filtered through gauze to remove all coarse particles of meat, etc., and the preparation of the vaccine is finished.

In order to be sure of the antigenic power of the vaccine we withdraw some c.c. from the culture after 24 hours, before the addition of formalin, centrifuge, and inject the clear fluid intravenously into mice to test for toxicity. If the lethal dose is less than 1/5 c.c. we discard the culture.

Vaccination.

This vaccine has been employed in small doses, 0·5 c.c. being given subcutaneously into the hind leg and we have recommended only one dose. Usually there is no reaction after the injection, but occasionally a slight passing lameness may be noticed. We recommend to keeping the vaccinated sheep indoors for the night following vaccination, as we have reason to think that rest is beneficial during the absorption by the tissues.

After a week the sheep will in most cases survive 1 c.c. of virulent culture subcutaneously, which kills a control.

Results.

We have now been employing this vaccine for three years, and the demand for it is rapidly increasing. In 1929 we issued 4,000 doses, in 1930, 80,000, and in 1931, 110,000 doses. Each parcel is accompanied by a report-form to be sent in by the farmer in question. Although we have not been able to get reports from all users we have got a sufficient number to show the efficiency of this vaccine. All the reports received are summed up in the following table, which shows the results for the three years our vaccine has been in use.

and at the same time for comparison the results with the vaccine prepared in Denmark, composed of dried culture + serum :—

1929	*Number of Vaccinated Sheep.*	*Losses from Vaccination.*	*Losses from Braxy.*	*Total Losses Per cent.*
Formolised vaccine	2,271	3	6	0·39
Danish vaccine	1,302	0	42	3·09
1930				
Formolised vaccine	25,202	119	190	1·22
Danish vaccine	4,448	0	99	2·23
1931				
Formolised vaccine	32,263	0	178	0·55
Danish vaccine	5,558	0	134	2·41

The comparatively high mortality from vaccination in 1930 was due to a batch infected with *B. sporogenes*, which was not noticed until long afterwards, having then caused considerable losses on some farms.

In the reports of 1931 a great many reporters have confounded the columns for loss from vaccination and loss from braxy, giving the losses from braxy in the column for loss from vaccination. This, however, does not cause much confusion as the reaction after vaccinations are in all reports given as none or negligible. The losses from vaccination may for 1931 be put at O, the few doubtful ones being counted with the losses from braxy.

In the table above the losses during the first week (except those caused by vaccination) are not counted, as immunity is not to be expected until a week after vaccination. Frequently the farmers do not begin vaccination until they are reminded of it by the beginning of the disease, which not seldom continues for some days after vaccination, then rapidly stops.

The great majority of our farmers vaccinate only lambs and yearlings, but on the worst " braxy-farms " the whole stock must be vaccinated every year. Aware of the risk of leaving young sheep unvaccinated, practically every animal is now inoculated, and so we have only relatively very few unvaccinated controls to compare with the vaccinated ones. In the reports for 1931 there are to be found a total of 127 unvaccinated lambs and yearlings. Of this number 52 were lost from braxy, or 40·9 per cent.

Several examples of the efficiency of this method of vaccination are recorded in the reports. In some places our vaccine has been employed with good results after the administration of the Danish vaccine had proved insufficient. The converse case is not reported. The susceptibility of unvaccinated animals is in many instances striking. An employee of one farmer had four lambs and one yearling among the farmer's vaccinated stock. He did not think it worth while to vaccinate these few animals. The result was that he

lost three of the four lambs and the yearling too from braxy. Another example : Two brothers, each with his own flock of sheep but both living at the same farm, report their experience of vaccination. One vaccinated his flock with our vaccine, the other left his flock unvaccinated. In the autumn the one who had not vaccinated began to lose one after another of his lambs and yearlings of braxy. Then he vaccinated his lambs with our vaccine. The disease then stopped in the lambs, but continued in the yearlings, until he a week later inoculated them also, whereupon the disease was definitely brought to stop. His brother did not lose a single sheep.

Summary.

Vaccination against braxy has been practised increasingly in Iceland since 1897. Dried cultures prepared by the Danish Serum Laboratory and our antiserum have been most used, on the whole with good results.

Since 1929 a new kind of vaccine, consisting of slightly formolised whole cultures, has been introduced by our laboratory and has given constantly better results than the Danish vaccine as shown by the vaccination reports. Although the vaccine contains a number of living spores the reactions are very slight and losses from vaccination negligible.

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AVIAN TUBERCULOSIS IN CATTLE IN GREAT BRITAIN.

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In this article an account is given of the isolation of strains of tubercle bacilli of the avian type from natural cases of tuberculosis in cattle in this country.

Mammalian animals, *e.g.*, swine, less commonly cattle, and very rarely sheep and horses, as well as mammals in the wild state, such as rabbits and mice, or wild animals in captivity, are known to acquire tuberculosis from contact with infected birds.

Plum (1925) in Denmark describes six cases of tuberculous abortion in cows belonging to six herds, in which the uterus and placenta were proved to be infected with tubercle bacilli of the avian type. Although measured amounts of culture were not tested, the virulence of the organisms appeared to be up to the standard for avian tubercle bacilli from fowls. More recently (1931) Plum has reported further cases of avian tuberculosis in cattle.

Schalk (1928) in the United States showed that by exposure to tuberculous fowls cattle could be made sensitive to avian tuberculin and that some of them develop small caseo-calcareous lesions in the glands of the alimentary tract.

Van Es and Martin (1930) examined material from 164 tuberculin-reacting cattle in which isolated tuberculous lesions, usually confined to the mesenteric lymphatic glands, were found at autopsy. In 115 of these cases it was possible to determine the type of the infecting organism, the result being that the strains from 100 cases were referred to a mammalian type, eleven to the avian type and in four both types were present. Bovine tubercle bacilli alone were encountered in the generalised lesions of 58 cattle examined.

Only one instance of natural avian tuberculosis in cattle has been recorded in Great Britain. This occurred in a calf (No. 1225) which had been used for an inoculation experiment with human tubercle bacilli in work for the Royal Commission on Tuberculosis. From a mesenteric gland of this calf A. S. Griffith (1911) isolated tubercle bacilli of the avian type.

The present observations, which are mainly concerned with cattle belonging to three herds, arose in the course of field work on the control of Johne's disease. This work involved periodic testing with johnin by the intradermal method, and from time to time selected animals were purchased for post-mortem examination for tuberculosis and Johne's disease.

It is necessary to refer briefly to the technique of this examination. The animals were usually slaughtered at a neighbouring abattoir, and after the carcase had been examined by the meat inspector the intestine and its associated glands, as well as the bronchial, mediastinal, submaxillary and pharyngeal glands, were brought to the Institute for more thorough investigation.

In the examination for Johne's disease the mucous membrane of the intestine was washed, and smears were taken from various parts for microscopic study. This was supplemented by cultural examination in which

fragments of the membrane from areas most likely to be affected were treated with antiformin and the material used for sowing culture media containing extracts of *Bact. phlei.* Cultures direct or after antiformin were also sown from some of the intestinal lymphatic glands. It is not proposed to describe in detail the technique used, but it should be stated that the methods adopted have been found reliable for detecting very small numbers of Johne's bacilli.

In the examination for tuberculosis, where naked-eye lesions were absent or where there was any doubt, material from the bronchial, mediastinal, submaxillary, pharyngeal and mesenteric glands was inoculated into guinea-pigs subcutaneously and intraperitoneally. Where there was reason to suspect that the animal was affected with avian tuberculosis cultures were made direct or after treatment with antiformin on glycerine egg medium, and inoculations of the suspected material were made to guinea-pigs and sometimes also to fowls. In this case the subcutaneously inoculated guinea-pig was killed after three to five weeks and material from the regional lymphatic gland and spleen was sown on glycerine egg medium. The intraperitoneally inoculated guinea-pig was killed and examined after two months. With guinea-pigs dying prematurely the regional lymphatic glands were inoculated to other guinea-pigs. Fowls were kept for two to six months and in some cases were tested at intervals with avian tuberculin.

History of the Cattle Examined.

Herd E.

This was a herd of the ordinary commercial type and was badly infected with Johne's disease and to some extent with tuberculosis.

Among the animals submitted to a johnin test on March 16th, 1931, were seven calves, of which two gave strong reactions and one a slight reaction. One of the former, C70, was bought for further examination.

C70 was 6½ months old at the time of purchase. A few days after it was bought the animal was retested on the opposite side of the neck with tuberculin and with johnin in dilutions of 10 and 100. Reactions occurred with both reagents at 1 : 10 dilution but were definitely stronger with johnin. (Details of the reactions given by C70 and by the other naturally infected animals are shown in Table I.) C70 was then slaughtered and post-mortem examination showed caseo-calcareous lesions resembling those of tuberculosis in the submaxillary and in several of the mesenteric lymphatic glands. The pharyngeal and bronchial glands, the lungs and other internal organs, were normal in appearance. The search for Johne's disease was negative. In sections of the mesenteric gland lesions scattered acid-fast bacilli were seen. These were isolated on glycerine egg medium by direct culture and by treatment with 10 per cent. antiformin, and they were also grown from the regional lymphatic glands of guinea-pigs inoculated subcutaneously with the original material. Acid-fast bacilli were also isolated through the guinea-pig from the submaxillary gland lesions. On subsequent examination the cultures were found to be composed of virulent avian tubercle bacilli.

On June 8th, 1931, some of the animals in Herd E were tested with avian tuberculin, and in a group of eight calves about six months

old no less than five gave strong reactions, including two (numbers 71 and 67) which had reacted at the previous test in March. Two of the reactors (C73 and C72) were purchased.

C73 was retested on July 1st, 1931, on the depilated skin of the back with avian tuberculin, johnin and tuberculin in dilutions of 1 : 10 and 100 (single injection only). Reactions to avian tuberculin were definite at 1 in 10 and slight at 1 in 100 ; the results with johnin were indefinite and there was no reaction to tuberculin. Eight days later C73 was slaughtered. The only lesions found were in the mesenteric glands, a number of these containing calcareous centres about twice the size of a pin's head, while in two glands there were calcareo-caseous masses as large as a pea and a hazelnut. Acid-fast bacilli, subsequently identified as virulent avian tubercle bacilli, were isolated from these glands by direct culture, as well as through the guinea-pig.

C72 was also tested on July 1st, 1931. Definite reactions, roughly of equal intensity, were produced by avian tuberculin and johnin at 1 : 10 and slight reactions with both reagents at 1 : 100, but with tuberculin there was no reaction. It was then decided to keep this animal for a time to determine whether the allergic condition would persist. Further tests with avain tuberculin, johnin and tuberculin were accordingly carried out on September 22nd, 1931 and December 28th, 1931 ; on both occasions strong reactions were obtained with avian tuberculin and definite reactions with johnin, and on December 28th, 1931, the reaction to tuberculin was also considered to be definite. On December 31st, 1931, the animal was slaughtered. A thorough search of the organs revealed no focal lesions suggestive of tuberculosis and the examination for Johne's disease was negative. Cultures from the glands, direct and by means of antiformin, and through the medium of guinea-pigs (two subcut. ; one died in 18 days and one was killed after 42 days) and a fowl (intramusc. ; killed in 180 days) yielded negative results as far as tubercle bacilli were concerned, but from the guinea-pig which died after 18 days all culture tubes were contaminated.

Herd F.

This herd consisted of about 20 cattle, mostly cows. Losses from Johne's disease had been heavy.

Among the reactors to a johnin test carried out on June 10th, 1930, was a yearling heifer (C19) which when tested three months previously had reacted to neither johnin nor tuberculin.

C19 was purchased in the expectation that she would be found to be affected with Johne's disease. She was kept under observation at the Institute for five months, and, although during this time her condition was rather poor, the fæces were normal in consistence. On September 1st,1930, and December 2nd,1930, she reacted strongly to johnin, and on September 1st, 1930, she gave what would probably be considered a slight reaction to tuberculin (*see* Table 1). C19 was slaughtered on December 16th, 1930, at an abattoir and the carcase was passed by the Inspector as free from tuberculosis. The intestine,

as well as the mesenteric, pharyngeal and bronchial glands, were brought to the Institute, where inoculation of guinea-pigs with gland material supported the opinion that C19 was not affected with bovine tuberculosis. The only noteworthy change in the intestine was slight congestion of the ileo-cæcal valve. In the search for Johne's disease scrapings from various parts of the mucous membrane were treated with 20 per cent. antiformin and the material sown on egg media with and without the addition of glycerine extract of *Bact. phlei.* To some of the tubes of *Bact. phlei*-egg medium gentian violet was added to a concentration of 1 in 10,000. By the thirteenth day numerous tiny waxy colonies had appeared on every slope sown (16 in number). On the tubes without dye colonies of two sizes developed, some being very small and others of the shape and size of split peas. The cultures were subsequently verified as being composed of avian tubercle bacilli of low virulence.

C75.—Among the reactors to johnin on March 30th, 1931, was a six months' old calf (C75). This animal was slaughtered 14 days later. Careful examination of the intestine and mesenteric glands showed no evidence of Johne's disease, but a number of the glands contained lesions suggestive of tuberculosis. In smears from these lesions acid-fast bacilli were seen, and these were isolated on glycerine egg through the guinea-pig following subcutaneous inoculation.

Herd G.

This herd, which was free from tuberculosis, consisted of 46 cattle, including 22 milking cows. From time to time animals had been lost through Johne's disease, and in two of these cases the diagnosis was verified by post-mortem examination. Among the animals reacting to a johnin test on May 12th, 1930, was a twelve-years' old cow (C18).

C18 was removed to the Institute, and as she was scouring and in poor condition she was slaughtered on June 30th, 1930. The carcase was emaciated and œdematous and was wholly condemned. Tuberculous lesions were not seen. Apart from bluish-red congestion (so-called " plum coloration ") of the ileo-cæcal valve, the intestine showed no changes suggestive of Johne's disease, and smears and cultures (direct and after treatment with antiformin) failed to show Johne's bacilli. The intestinal glands appeared to be enlarged and on section an excess of milky fluid drained away, but there was no focal disease. No acid-fast bacilli were seen on direct examination of the gland juice, but after treatment with antiformin centrifuge deposits showed a few clumps of acid-fast bacilli which were considered to be rather longer and thicker than typical Johne's bacilli. Four tubes of egg medium (with and without the addition of *Bact. phlei* extract) showed growths which were identified as consisting of avian tubercle bacilli of low virulence.

As soon as the nature of the infection in C18 was established, the young stock on the farm, including eight animals less than twelve months of age, were tested with avian tuberculin. Of 19 animals

tested only one reacted, but, though this animal could not be obtained for further examination, the result indicated that the great majority at any rate were not infected with avian tubercle bacilli.

TABLE I.

INTRADERMAL TESTS WITH AVIAN TUBERCULIN, JOHNIN AND ORDINARY TUBERCULIN.

			Avian Tuberculin		*Johnin*		*Tuberculin*	
Herd.	*Animal.*	*Date tested.*	*Pure.*	10	*Pure.*	10	*Pure.*	10
E	Calf 70	16.3.31			21 VP 30 (n.r.) 24 VP 35			
		25.3.31				12 P diff. 16 P diff.		10 12 VSP
	Calf 73	8.6.31	18 P. 30 × 30 (n.r.) 22 P					
		1.7.31		18 P diff. (n.r.) 19 SP 40		11 (n.r.) 12 S oed.		8 (n.r.) 8
	Calf 72	8.6.31	19 P 30 × 30 (n.r.) 18 P					
		1.7.31		11 S oed. (n.r.) 10 20 diff.		12 S oed. (n.r.) 11 S oed.		9 (n.r.) 9
		22.9.31	11 SP S. oed 16 VP 30 × 20 diff.		7 11 P diff. oed.		7 10	
		28.12.31	15 SP 20 25		11 S oed. 16 15		12 S oed. 13 diff.	
F	Calf 75	30.3.31			18 P 50 × 35 (n.r.) 22 P 30			
	Cow 19	10.6.30			22 SP 50 × 40 (n.r.) 25 P 70 × 40			
		1.9.30			18 50 × 30 diff. 22 P 30 diff.		12 17	
		2.12.30			15 SP 20 22 SP 25	13 SP 15 16 SP 20	10 12	
G	Cow 18	12.5.30			17 21 P 80 × 40			
Experimental Cow 27 (inoc. 2.1.31 with strain C 18)		28.12.30	10 10					
		24.1.31	22 P 45 × 35 36 VP 60 × 60		20 P 30 × 30 34 VP 50 × 50		11 14	
		10.3.31	16 23 P		12 18 SP		10 14 VSP	

NOTES.

(1) "Pure" = reagent conc. to 1/10 orig. vol., except with C 27 where the "pure" tuberculin was conc. to 1/5 orig. vol.; "10" = "pure" dil. 1 in 10.

(2) The amount injected was 0·2 c.c., except with Calf 72 (all reagents, 22.9.31 and 28.12.31) when 0·1 c.c. was used.

(3) Normal skin measurements were about 5 mm. in calves and 7 mm. in cows.

(4) The first line shows the reaction at the 48 hour and the second line that at the 72 hour, a second injection being made at the 48 hour except where stated (n.r.) = "not re-injected." The first figure gives the thickness of the double fold of skin in mm. and the second figure or figures indicates the estimated diameter or surface measurements of the swelling. "P" = painful; "V = very; "S" = slightly; "oed" = œdema; "diff." = diffuse.

(5) The johnin was prepared by A. L. Sheather from cultures on Sauton's synthetic medium containing *Bact. phlei* extract, using a strain of Johne's bacillus originally isolated by G. W. Dunkin.

The avian tuberculin was prepared by A. L. Sheather from an avian strain originally isolated from a fowl. For tests of 8.6.31 the reagent was prepared from cow 18 strain.

The tuberculin was obtained from A. L. Sheather, except that used on 28.12.31 which was prepared in the Dept. of Animal Pathology, Cambridge.

The Source of Infection.

Inspection of the poultry in Herd E showed that some of the fowls were unthrifty, and the owner stated that during the previous year or two there had been a number of sudden deaths among them. The birds were allowed to roam freely about the cattle sheds and yards. Three fowls in poor condition were killed, and on autopsy extensive lesions of tuberculosis were found in the liver and spleen, and in one case there was ulceration of the intestine. The nature of the disease was verified by bacteriological examination, including the inoculation of fowls and rabbits.

In Herd F the farm manager was not aware of any serious disease among the poultry, but some of the fowls were thin, and they had free access to the pastures and buildings occupied by the cattle. In November, 1931, 20 rather old and unthrifty fowls were tested in the left wattle with 0·05 c.c. concentrated avian tuberculin. Seventy-two hours later there were nine definite reactors and of these three were killed for examination. The spleen of each bird contained yellowish-white lesions, 5 to 10 mm. in diameter, which on section were irregular in outline and rather firm in consistence. Cultures of avian tubercle bacilli were obtained from each bird.

In Herd G the poultry flock was not specially examined for tuberculosis, but the birds appeared to be in good condition, and there was no history of infectious disease among them. They had free access to the cattle yards and sheds. C18 had been in the herd for about eleven years, and no light could be thrown on the source of her infection.

Bacteriological Investigations.

Cultural Characters.

In primary cultures on *glycerine egg* numerous small pearly-white colonies appeared as early as the twelfth day and were often most numerous on and about particles of the seed tissue. Colonies were smooth, raised and moist to the touch and later became creamy-yellow. When well isolated they might attain by the end of the second month a diameter of 2 mm. or even more. On the larger colonies tiny knobs or excrescences were liable to develop and these sometimes grew out into folds or convolutions. Subcultures on this medium sometimes showed a slight tendency towards wrinkling. As is well known, the characters just described are typical of avian tubercle bacilli. The stimulating effect of glycerine, when added to media in a concentration of 5 per cent., was striking. On *plain egg* growth was much slower than on glycerine egg, consisting at first of a thin layer not easily seen, except with strain C18 which grew more abundantly. During the second month isolated pearly colonies developed on this thin background. On *glycerine agar* the strains grew as moist continuous layers of irregular thickness, at first greyish and later faintly brownish. During the third month cultures of strains C 70, 18 and 19 on this medium were somewhat wrinkled.

On glycerine potato cultures developed into heavy moist and creamy growths with smooth surfaces, which later became bosselated. In tubes of 5 per cent. glycerine broth a slimy layer developed at the bottom of the clear medium ; with strains C70 and 75 after three to four months a smooth whitish surface film appeared which became irregularly thickened but not wrinkled. On *solid serum* strain C18 gave a thin, transparent moist growth, while a 30-day culture of the same strain on serum agar was noted to be coherent and not easily emulsifiable. Cultures of strains C70, 75 and 19 on glycerine agar and glycerine egg grown at 37° C. acquired a deep orange colour after being placed at room temperature for a few weeks.

In one instance a primary culture on glycerine egg from a guinea-pig inoculated with strain C19 contained a mixture of smooth pearly colonies about 1 mm. in diameter and colonies twice as large with rough surfaces and irregular edges. (By subcutaneous inoculation of a guinea-pig it was ascertained that the rough colonies contained no virulent mammalian tubercle bacilli.) Petroff and Branch (1929) and Petroff and Steenken (1930) have also reported the dissociation of cultures of avian tubercle bacilli into smooth and rough forms.

Microscopic Examination.

Young primary cultures on glycerine egg were composed of short rods uniformly and strongly acid-fast by the Ziehl-Neelsen method. In older cultures on various media the organisms were pleomorphic, appearing as rods or as short filaments sometimes of variable thickness and with clubbed extremities. For the most part resistance to decolorisation with 25 per cent. sulphuric acid was strong, but uneven staining was often seen in the longer rods and this was sometimes particularly prominent on glycerine egg media. In some cultures a proportion of the organisms were decolorised by the acid and took the blue counterstain, and, in the manner recognised to be characteristic of avian tubercle bacilli, the contrast staining was often uneven, and the organisms then presented the appearance of a string of tiny cocci or very short rods.

Pathogenicity.

Tests were made on guinea-pigs, rabbits, fowls, goats, sometimes on mice and on one occasion on a cow. The larger animals were inoculated as much with the object of studying the allergic condition produced as of studying the pathogenic properties of the cultures. In most cases weighed amounts of culture were used in order to obtain an estimate of the virulence, the growth being taken from three to four-week glycerine egg cultures, not more than three generations removed from the body. From the results summarised below it may be concluded that the strains from C70, 73, 75, 19 and 18 belong to the avian type of tubercle bacillus and that the strains from the two cows were of decidedly lower virulence than those from the calves.

Guinea-pigs.—Subcutaneous inoculation of the original material (submaxillary or mesenteric gland) either produced no visible effects,

or tuberculous changes were limited to the point of inoculation and the adjacent gland. Strains C70, 73 and 75 were isolated from the inguinal glands of guinea-pigs in this way (those from C70 and 73 also by direct culture), but with strains C73 and 75 the local glands showed slight enlargement only.

Two guinea-pigs injected subcutaneously with mesenteric gland material from C 19 and 18 died after eight and 14 days and cultures were not made ; two guinea-pigs injected intraperitoneally with the same material showed no lesions when killed after 60 days.

With all five strains 1·0 mg. of culture, injected subcutaneously, produced no disturbance in the general condition, and changes were almost wholly confined to the site of inoculation and the neighbouring glands when the animals were killed 41 to 97 days later. Tubercle bacilli were present in the lesions.

Rabbits.—Strains C70, 73 and 75 were inoculated intravenously into ten rabbits altogether, in doses ranging with each strain from 2·0 to 0·01 mg. The animals lost weight considerably and died within nine to 57 days, the duration of life in four rabbits receiving 1·0 mg. being from 17 to 26 days and in three rabbits receiving 0·01 mg. from 33 to 57 days. Post-mortem examination showed acute tuberculosis of a nature recognised to be characteristic of infection with virulent avian tubercle bacilli. The effects were more or less similar in all cases and the following is an example.

A rabbit inoculated with 0·01 mg. 22-day glycerine egg culture of strain C 70 died in an emaciated condition 37 days later. At autopsy the spleen was considerably enlarged and contained a moderate number of just visible grey foci ; the liver showed a few small white nodules ; the lungs were crepitant but somewhat œdematous and showed scattered over the surface a large number of hæmorrhages varying from minute points up to 2 mm. and greyish tubercles up to 1 mm. in size. There were also a few tiny nodules on the peritoneum covering the kidneys. Smears from the liver and spleen showed very large numbers of acid-fast bacilli, many of them accumulated within cells.

The strains from C19 and 18 were decidedly less virulent for rabbits and in doses of 1·0 to 0·01 mg. intravenously produced suppurative lesions about the joints.

As examples, two rabbits (R1 and R2) inoculated intravenously with 1·0 mg. amounts of 22-day glycerine-egg cultures (third generation) died in an emaciated condition after 124 and 163 days. The most prominent lesions in both animals were in the joints of the forelimbs, those in R1 being in the shoulder and carpal joints and in one elbow, while those in R2 were in the elbow and carpal joints. Both rabbits also showed slight lesions in the kidneys, and R2 showed three firm nodules in the liver and caseation of the lower margins of the lungs. A third rabbit which received 0·01 mg. of the same culture of strain C19 had gained 590 gm. in weight when killed after 187 days, and in this case the only visible lesions was situated in the right carpus. From all three animals, however, glycerine egg cultures with the characteristics of avian tubercle bacilli were obtained from the joint pus and from the spleen.

The difference in virulence between the calf and the cow strains was also apparent to some extent when 1·0 mg. amounts of culture were injected into rabbits subcutaneously on the back.

For example, a rabbit which received strain C75 in this way had lost 510 gm. at the time of death after 79 days and post-mortem examination revealed at the site of inoculation a perforation of the skin and a flattened cavity about 3 cm. across containing caseous pus. The nearest lymphatic gland was softened and necrotic ; the lungs showed a few small grey nodules with white centres, and there were five small yellowish-white nodules up to 2 mm. in one kidney, in which acid-fast bacilli were seen microscopically. The spleen, which was quite small, the liver, testes and joints showed no lesions. A rabbit which received 1·0 mg. C18 culture was killed on the 184th day after having gained 710 gm. and at autopsy there was a flat oval sac-like swelling containing pus, from which acid-fast bacilli were cultivated. No other lesions were present and the spleen was culturally negative.

Fowls.—Altogether six fowls were inoculated intravenously with cultures from strains C70, 73 and 75, 1·0 and 0·01 mg. being the amounts used with each strain. The duration of life ranged from 31 to 41 days, except one bird which after receiving 1·0 mg. (strain C75) was killed 73 days later when very thin and anæmic. The immediate cause of death in one case (0·01 mg., strain C70) was rupture of the spleen. One bird inoculated with 2·0 mg. of strain C70 died in 21 days. All had lost considerably in weight and showed enlargement of the liver and spleen, in which numbers of just visible grey tubercles could sometimes be detected. Smears from these organs showed acid-fast bacilli in enormous numbers, many of them bunched within cells.

Three fowls inoculated into the pectoral muscles with strains C70 (2·0 mg.) and 75 (2·0 and 1·0 mg.) died in an anæmic condition in 49, 45 and 54 days, death in the first instance being directly due to rupture of the liver. In each case there was a yellowish caseous lesion at the point of inoculation in the muscle, and the spleen and liver were filled with small grey tubercles. Tubercle bacilli could be demonstrated in all these situations.

Tests on fowls supported the experiments on rabbits in showing that the strains from C19 and 18 were of low virulence.

Thus, a fowl receiving intravenously 0·01 mg. of a C19 culture had gained 400 gm. and was in fat condition when killed after 187 days, and post-mortem showed in the liver about six whitish nodules up to 4 mm. Tubercle bacilli were cultivated from the liver and spleen. A fowl receiving intravenously 1·0 mg. of a C18 culture was in good condition when killed after 184 days ; no lesions could be found and tubercle bacilli could not be recovered from the tissues. Another fowl, however, inoculated intravenously with 0·01 mg. of the same culture and killed after 184 days had one lesion in the spleen as large as a pea, which on section showed concentric rings, and about a dozen similar lesions in the liver, from which tubercle bacilli were cultivated.

Feeding tests with 10·0 mg. amounts of culture by means of a

blunt pipette introduced into the œsophagus gave the following results.

A fowl fed with the more virulent strain C70 was thin and weak when killed after 184 days. The spleen contained cheesy nodules, the dimensions of peas, which could be easily shelled out from the surrounding soft tissue, and there were about a dozen similar nodules in the liver. Tubercle bacilli were cultivated from the lesions in both situations. In the wall of the intestinal canal two lesions were found ; the first the size of a split pea contained cheesy material and was situated about halfway through the canal, while at the entrance to the cæca there was a similar lesion in which a small number of long acid-fast bacilli were found microscopically.

A fowl fed with strain C73 had gained 330 gm. and was in good condition when killed after 195 days. The liver was pale and contained about a dozen dirty-white tough centres up to 2 mm., easily shelling out, and the spleen contained two similar lesions. From both organs tubercle bacilli were cultivated. In the intestinal wall at two different points lesions, necrotic in appearance and rather smaller than peas, were found, and in both of them long acid-fast bacilli were demonstrated in small numbers.

A fowl fed with strain C75 was weak and emaciated prior to its death after 72 days. In the crop there was a caseous lesion the size of an oatgrain.

By contrast, two fowls fed with cultures of strains C19 and 18 remained in good condition until they were killed after 187 and 184 days. Careful search of the intestine and the organs failed to show lesions and no tubercle bacilli could be cultivated.

(No attempt was made to see whether the virulence of strains C18 and 19 could be raised by passage through fowls or rabbits.)

Seven fowls, which were allowed to survive 184 days or more after being fed or inoculated with strains from C70, 73, 19 and 18, were tested in turn with avian tuberculin, johnin and tuberculin by injecting into the wattle 0·1 c.c. of the concentrated reagent after dilution to its original strength. The interval between the tests was not less than 20 days and the injections were made into the wattles alternately. The results indicated that infection had ensued in five of the seven birds, the two exceptions being birds which had been fed with 10 mg. culture of strains C19 and 18. In four of the five cases avian tuberculin gave a stronger reaction than johnin or tuberculin, and in three of the four cases the reaction to johnin was stronger than that to tuberculin. The fifth bird was highly sensitive to all three reagents (œdematous infiltration rendering the wattle four or five times the normal thickness for at least three days). In four cases the sensitive state persisted for three months and in one case for over six months.

Mice.—A few inoculations subcutaneously and intraperitoneally with quantities of about 0·25 mg. showed that the virulent calf strains could survive in the spleen and liver for periods of at least three months, though death was not always produced within this time. Even when these organs were not visibly altered, it was sometimes possible to demonstrate tubercle bacilli in smears or cultures. Occasionally (strains C70 and 75) the spleen was definitely enlarged and swarming with bacilli, and in one instance the lungs were studded with tiny grey tubercles.

Goats.—One adult non-lactating female was inoculated subcutaneously in the neck or thigh with 5 or 10 mg. culture of each strain. This produced an abscess which discharged through the skin, and the neighbouring gland attained the size of a hen or turkey egg, receding later. Apart from this, the animal remained outwardly in normal health. The goats inoculated with strains C70, 73 and 18 were killed after 126, 169 and 109 days. By this time the local lesions had practically disappeared and, though not enlarged, the local glands contained caseous lesions the size of peas or cobnuts, from which tubercle bacilli could be isolated. The two goats inoculated with strains C75 and 19 were killed after 174 and 241 days, and in both cases the local changes had disappeared and tubercle bacilli could not be cultivated. No tuberculous lesions were seen in the internal organs of any goat, special attention being paid to the uterus and udder.

Two adult goats were fed each with two whole glycerine egg cultures of strains C19 and 18. They showed no ill effects, and when killed 62 days later no lesions could be seen in the throat glands or elsewhere.

At intervals varying from one to eight months after inoculation the goats were tested intradermally with avian tuberculin, johnin and tuberculin in dilutions of 1 in 10, 100 and 1,000. Reactions were strongest with avian tuberculin and in many cases reached the 1,000 dilution ; reactions with johnin were of intermediate intensity, being appreciable usually to the 100 dilution, while with tuberculin the reactions were comparatively feeble, rarely extending beyond the ten dilution. A similar gradation of reactions was observed with two goats inoculated as controls with avian strains from a fowl and from a pig. The sensitive state persisted until the animals were killed four to five months after inoculation, but the goat injected with the less virulent strain from C19 was kept alive for eight months, and at the end of that time it was still highly sensitive. The goats fed with the avian cultures from cattle did not react.

Cattle.—A cow, C27, which did not react to tuberculin or johnin, was injected subcutaneously in the neck with 5·0 c.c. of a moderately hazy emulsion from a month-old glycerine potato culture of strain C18. This caused a somewhat painful local swelling, but there was no abscess formation and no appreciable enlargement of the prescapular gland. The cow was in good general health until slaughter 80 days later. On autopsy C27 showed practically no local lesion and no enlargement of the regional gland, although cultures of the injected organism were obtained from both places. The examination for Johne's disease was negative and no lesions were seen in the internal organs.

At various times after inoculation the cow was tested with avian and mammalian tuberculin and with johnin, with the results shown in Table I. It will be seen that C27 reacted strongly to avian tuberculin and johnin and slightly to ordinary tuberculin.

Discussion.

The characters of the tubercle bacilli isolated from the five animals in Herds E, F and G clearly correspond with those of the avian type. The disease was obviously acquired by the alimentary route, and it was proved that in Herds E and F the cattle were in direct contact with tuberculous poultry. There was evidence that in Herd E at any rate infection was not confined to the animals selected for autopsy, and the failure to discover infection in one of the calves from this herd is not surprising in view of similar failures by other investigators, *e.g.*, Plum (1931).

There is no accurate information as to the incidence and distribution of tuberculosis in the poultry flocks of Great Britain, but the disease is certainly well known, and hence there is reason to believe that infection of cattle in this country must be more frequent than is suggested by the almost entire absence of recorded cases. Diseased poultry are, no doubt, the main direct source of avian tuberculous infection in mammals. It is known, however, that bovine tubercle bacilli appear in the milk of experimentally inoculated cattle and goats. In the case of avian tubercle bacilli, the long persistence of the organisms in the milk of an inoculated goat has been recorded by A. S. Griffith (1927), and recently avian tubercle bacilli have been found in the milk of cows belonging to a few small herds by Wolters and Dehmel (1931, 1932). It is not impossible, therefore, that calves may sometimes be infected through the milk.

Although natural cases of avian tuberculosis in mammals with disemminated lesions are known to occur occasionally in the pig, and although single instances of this have been described in the ox by O. Bang (1908), in the sheep by A. S. Griffith (1925), and in deer by Magnusson (1923) and Christiansen (1931), the disease in mammals is generally localised and retrogressive and therefore not in itself serious. The two cows in the present series are of interest in that one of them was very old and that although a careful search of the mesenteric glands failed to reveal lesions of microscopic size these organs were proved to be infected; in the second animal avian tubercle bacilli were isolated from the apparently healthy intestinal mucous membrane, recalling the case of avian tuberculosis in a horse described by McFadyean in 1918, except that in this case the mucous membrane of the intestine was thickened in places. Cows 18 and 19 were the first cases met with and, as at that time avian tuberculosis was not suspected, unfortunately no histological examination was made of the intestine or glands.

Of the five strains isolated, the three from calves produced acute and characteristic disease in rabbits and fowls in small doses intravenously and therefore were not definitely below normal virulence. On the other hand, the strains from the two adult cattle were of quite low pathogenicity for rabbits and fowls. This suggests that, without any question of modification of type (*cf.* F. Griffith, 1911), avian tubercle bacilli may lose virulence as a result of a more prolonged

residence in bovine tissues, just as they sometimes appear to do in the pig and as bovine tubercle bacilli are known to do in the horse. It is further possible that avian tubercle bacilli thus attenuated may become still better adapted to life in the tissues of the bovine host, in the way that A. S. Griffith (1928) considers may happen with bovine tubercle bacilli in the horse. If this is the true explanation with C18 and 19 and if such incomplete or delayed recovery is at all common in avian tuberculosis in cattle, the matter would have some practical importance, because such animals would presumably continue during adult life to give cross-reactions with johnin and perhaps also with tuberculin. It is not proposed to discuss the question of non-specific reactions to avian and mammalian tuberculin and to johnin, but the observations recorded in this paper agree with those of Plum (1925) in showing that animals naturally infected with avian tuberculosis may respond to intradermal tests with johnin as well as with avian tuberculin. It is also evident that such cross-reactions are not confined to calves.

Schalk (1928) found that cattle lost the power to react to avian tuberculin two to five months after their removal from the vicinity of tuberculous fowls, and Plum (1931) has similarly reported that six months after cattle are removed from the source of infection the reactions are markedly diminished. That this may not always be so is shown by the examples of C72 and 19. These animals were removed for six months from any opportunity of reinfection, but after this interval their reactivity was as high as at the first test (Table I). Moreover, the post-mortem findings on one natural case, C72, as well as on the goats inoculated with C75 and 19, give some grounds for the suggestion that the sensitive state may continue for a time after the tissues have overcome the infection.

Summary.

The natural occurrence of avian tuberculosis in cattle in Great Britain is reported. The nature of the infection was established in three calves and in two adult bovines, belonging to three herds, but it was probable that other cases among calves existed in at least one of these herds. In two of the herds tuberculosis was proved to exist among the fowls, and these had free access to the cattle.

In four instances the organisms were isolated from glands of the alimentary tract and in the fifth instance from the apparently normal intestinal mucous membrane.

Four of the five cases were detected as a result of reactions to the intradermal johnin test, but, where tests were made, the animals reacted still more strongly to avian tuberculin and in a smaller degree to mammalian tuberculin. Post-mortem examination, however, failed in all cases to show the presence of bovine tuberculosis or Johne's disease. With two animals strong reactions to avian tuberculin or johnin could be elicited for a period of six months after removal from the source of infection, but at the time of slaughter one of these had apparently overcome the infection.

The strains of avian tubercle bacilli from the calves were highly virulent for rabbits and fowls ; those from two adult cattle were of low virulence, probably owing to a more prolonged residence in mammalian tissues.

The practical importance of avian tuberculosis in cattle is discussed.

I am indebted to Dr. A. L. Sheather for supplies of avian tuberculin, johnin and tuberculin, and to Professor J. B. Buxton for a supply of tuberculin.

Note.—On the completion of this work, the strains of tubercle bacilli from C70, 73, 75, 18 and 19 were submitted to Dr. A. Stanley Griffith, who kindly informs me that they were all agglutinated by a serum prepared by him with a strain of avian tubercle bacilli from a fowl, and completely absorbed the agglutinin from this serum.

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REVIEWS.

Die Rachitis in ihren aetiologischen, biologischen, pathogenetischen, pathologisch-anatomischen, und Klinischen Beziehungen. **Biochemischer Teil.** Von Dr. Oskar Wellmann. Gustav Fischer, Jena, 1932. Preis, 25 Rmk.

This work is complementary to the one previously published (1931) by Porfessor Marek, which was reviewed in the previous volume of this Journal (Vol. XLIV, p. 154). It gives the results of Professor Wellmann's experimental Studies of Rachitis from the chemical standpoint, which were begun as long as 1912 and had to be interrupted for a period during the recent great war. It comprises 480 Royal 8vo pages, which gives in minute detail an account of the experiments as regards the chemical analyses of the tissues of the animals used, of their diet, and of their excretions during the period of experiment. It is on the precise information thus gained that the author founds his conclusions regarding the effect of errors in the composition of different diets in causing rachitis. In this connection the most important points are as follows :—

In estimating the effect of different kinds of diet the author considers it useful to calculate the alkaline earth basicity, or EA. This is defined as the difference between the alkaline earths and the phosphoric acid (expressed in milligram equivalents) in 100 gm. of the dry substance.

A definite proportion must be maintained between the calcium and the magnesium, and the magnesium must not exceed a third of the amount of calcium. It is important that the ash of the diet should be weakly alkaline or positive EA, the optimum degree of the latter being plus 20—25 mg. equivalence. Deviations towards either the positive or the negative side produce rachitic disease.

Other investigators have maintained that the Ca/P proportion in the diet is alone decisive and therefore require a value of 2. Although in this way one can form an approximately correct conclusion regarding the tendency of a diet to produce rachitis, the author maintains that in this respect the EA criterion provides a more certain basis.

Thus, in the author's own experiments in which the animals were protected against rhachitic disease, the Ca/P proportions varied between 1·81 and 3·52, while the EA was of the optimum value of + 20 to 26 mg. equivalence. Besides, the numerical value of the Ca/P proportion was always affected by the different constituents of the food mixture ; for example, through a preponderance of bran it was lowered, and through rice poor in magnesium it was raised. With an optimun EA in certain circumstances a bran mixture had a Ca/P proportion of 1·68 and a rice mixture one of 4·93 in spite of the fact that in both cases the food mixture did not represent a rachitis-producing diet.

The author holds that his own results unquestionably show the decided importance of an absolute calcium and phosphorus deficiency, an inappropriate EA, or a proper quantity proportion between alkaline earths and phosphorus for the genesis of rachitis.

The advantage of an alkaline character of the diet was also supported through the further observation that an o-value EA does not suffice completely to prevent rachitic disease. Consequently, the view hitherto almost generally held that rachitis can only be produced through absence of phosphorus is not correct, for the author's own experiments showed the same importance of a calcium deficiency or of an inappropriate proportion between the earthy alkalies and phosphoric acid. Further, the experimentally produced rat rachitis stands in causal connection with an inappropriate quantity proportion of calcium and phosphorus, especially with a too strongly positive

EA, as has been especially shown in the experiments of McCollum and of Steenbrock and Black.

According to a further observation the rachitis producing effect of an absolute or proportionally insufficient provision of minerals can be corrected by an at least 50 per cent. ultra-violet irradiation of the food, or by the administration of the D-vitamin. With regard to the mode of action of the D-vitamin, it may be assumed that it regulates the Ca and P exchange, increases the assimilation of the mineral stuffs, diminishes their excretion by the intestine, and favours the excretion by the kidneys of the mineral ingredients which disturb the acid base equilibrium.

The decisive importance of a corresponding completion of the mineral stuff in the diet was shown by the following observations. Whereas completion with carbonate of lime up to the production of an optimum EA absolutely hindered rachitic disease, a food mixture completed by the addition of phosphoric acid 2 r. with carbonate of lime up to o-value EA was not fully anti-rachitic in spite of the fact that in such an animal there was a D-vitamin addition. On the other hand severe rachitis was brought about through a food mixture completed by magnesium carbonate, instead of calcium carbonate, up to an optimum positive EA of +22·98 mg. equivalence, because its absolute calcium amount was insufficient. This also shows that in the formation of bone the calcium cannot be replaced by magnesium, and that the magnesium quantity should not exceed a third of the calcium, because otherwise the superfluous magnesium still increases the excretion of Ca. and P., and in this way tends to produce rachitis.

It was shown by experiment that, with an absolutely sufficient amount of calcium in the diet, the production of a negative EA through an Na_2Hpo_4 addition does not have rachitic effect because, owing to the firmer combination of the sodium (in comparison with calcium) with the phosphoric acid, it is in a large measure excreted unchanged with the urine without any disturbance of the calcium and phosphorus metabolism.

In conclusion, it may be noted that all the experiments were carried out with young pigs, a fact which probably makes the results more directly applicable to the other animals of the farm, and to man, than some of those obtained with rats.

The work, of course, is not for either the student or the practitioner, but it ought to be found in every library in which books on medicine, biology, and chemistry are supposed to find a place.

Animal Diseases in South Africa. By M. W. Henning, Professor of Veterinary Science in the Faculty of Agriculture University of Pretoria. Vol. I, Bacterial and Protozoal Diseases. Vol. II, Virus and Deficiency Diseases, and Plant Poisons. South Africa Central News Agency, Ltd., 1932. Price, £2 10s.

This work extends to 878 pages, nearly equally divided between the two volumes, and it has 127 figures in the text.

With few exceptions, the bacterial diseases described are not special to South Africa, and include anthrax, tetanus, contagious abortion, tuberculosis, glanders, etc. The protozoan diseases dealt with are special to South Africa, or at least not found in Europe. Vol. II is about equally divided between the virus diseases and diseases caused by poisonous plants. Following the poisonous plants there are three pages under the heading " Deficiency Diseases." The virus diseases are mostly South African, but they include rinderpest, contagious pleuro-pneumonia, swine fever, canine distemper and rabies.

When the author started to compile a work on diseases of animals in South Africa, he had lying ready to his hand, in the series of Annual Reports by the Director of Veterinary Research, a valuable source of information regarding the diseases that are peculiar to, or of outstanding importance in that part of the world, and he deserves to be congratulated on the use he has made of that material. The task was made much more difficult when he decided to include diseases which have had a nearly world-wide occurrence, and a literature that in time and space has been much more scattered, and is therefore less easy to summarise or condense. That no doubt partly explains the fact that the author's account of these older diseases is less satisfactory, but it must regretfully be said that there are in this part of the work errors indicating slight acquaintance with either the diseases or the literature relating to them.

The following are examples of such unexpected errors :—

Anthrax.—The bacillus of anthrax " passes from the dead carcase into the soil, where it maintains its vitality for long periods, as a result of which certain pastures become notoriously infected " . . . " As long as an infected carcase is left unopened the bacilli remain unchanged." As every one who has had much experience with the disease must know, anthrax-bacilli in the unopened carcase almost immediately become changed, and, in warm climates almost the whole of the bacilli in the carcase may have suffered degeneration and actual disintegration within 24 hours after death.

Later, it is stated that in the areas like the Karroo, which are devoted almost exclusively to sheep farming, anthrax is rarely met with, " the arid nature of the veld being apparently unfavourable for the propagation of the virus." The author here appears to have formed a very exaggerated estimate of the saprophytic multiplication of the anthrax bacillus in soil. Very serious losses have been caused by anthrax on land as arid as the Karroo.

In acute glanders, it is said, a nasal discharge and swelling of the submaxillary lymphatic glands usually appear after an incubation period of about two to four days, the nasal discharge being yellowish-green or bloody, ichorous. One may venture the opinion that no previous author has ever recorded such a short period of incubation in the naturally contracted disease.

In illustration of the pulmonary lesions of glanders there is a figure which reproduces the flat surface of a slice of lung. The appearance of the lesions in it is so unusual as to suggest an error in diagnosis.

On the subject of glanders in man, it is observed that in the chronic cases " occasional recovery has followed the superficial application of ointments like grey mercurial ointment."

In the article on strangles it is said that foals " are very rarely born with uterine infection of this disease."

To explain the occurrence of strangles in a locality after an absence of several years, and when there is no evidence of its introduction from elsewhere, " it is considered that streptococci, which normally live as facultative parasites, suddenly invade the system of a horse whose resistance has been lowered by outside influence, and set up symptoms of strangles. As a result of the infection the virulence of the organisms becomes sufficiently exalted to cause infection of several other susceptible horses, and an outbreak of strangles." That appears to be an original opinion, but a more probable one would be that evidence had been overlooked.

In the article on tuberculosis it is said " There exists no positive evidence to prove that the progeny of a tuberculous male is infected at the time of birth." It is very difficult to extract any sensible meaning from this sentence. It appears to convey the idea that the progeny of a tuberculous male is never

infected at the time of birth, which, of course, is absurd, since, as is generally admitted, in cases of congenital tuberculosis the infection is usually derived from the female, and there exists no positive evidence to prove that a tuberculous male cannot impregnate a tuberculous female.

The following is the author's description of the histology of a tubercle. " If a microscopic examination of the tubercle is made the characteristic giant cells, several containing tubercle bacilli, will usually be found without much trouble. Numerous small round cells are sometimes also seen."

In the account of tuberculosis in horses the frequency with which the spleen is the seat of lesions is not mentioned, nor is the involvement of the bones of the vertebral column. In describing the lesions in the pig no mention is made of the spleen, although that is one of the common sites of the lesions.

The account given of tuberculosis of the udder is absolutely misleading. It is said " When the udder is affected large firm tuberculous abscesses are formed in the mammary tissue, and numerous small yellow tubercles are scattered through the rest of the gland."

The late Professor B. Bang, 47 years ago, gave a description of the lesions in tuberculosis of the cow's udder which has become classical and has been generally admired for its accuracy by all those who have restudied the disease. One is, therefore, entitled to protest when any author, without giving reasons for his dissent, seeks to import into the literature on the subject an entirely different account of the disease.

The Principles of Animal Hygiene and Veterinary Medicine. By Leunis Van Es, V.S., M.D., Professor of Animal Pathology and Hygiene in the University of Nebraska. New York: John Wiley and Sons, and Chapman and Hall, London, 1932. Price 40s. net

The title of this work hardly conveys a correct idea of its scope. Excluding the Index, it has 746 8vo pages, of which the first 332 deal in a general way with such subjects as soil, air, water, food, climate, buildings and disinfectants, while in the remainder all the important contagious diseases of the domesticated animals are dealt with in separate chapters with regard to etiology and prevention. The work appears to justify the author's hope that it may prove useful to students in schools and colleges as well as live-stock sanitarians and those engaged in animal husbandry. Upon the whole it gives a good exposition of the present state of knowledge and opinion regarding the contagious diseases from the point of view of prevention, and the author has not lost sight of the fact that in some cases, tuberculosis, for example, a method that gives good results in one country may be inadvisable or impossible in another. One of the least satisfactory chapters is the one headed " pig Typhus." This is said to be the disease of which Salmon and Smith discovered the cause—*B. suipestifer*. It is held accountable for great losses among young pigs, up to four months old, but older swine are sometimes attacked. It is characterised by intestinal ulceration, and apparently in the United States bacillary hog-cholera is another name for it. It is more particularly attached to certain farms and breeding establishments. The bacillus is an inhabitant of the intestine of normal swine. On farms where the disease is a perennial problem it is suggested that immunisation of the young pigs might be attempted as an experiment; using a vaccine or bacteria from *B. suipestifer* or *B. paratyphosus* strains. All this might have been written before the discovery of the filter-passing virus of hog-cholera, but it is now very misleading when it is not accompanied by a warning that pigs showing the symptoms or the lesions of this so-called ' pig typhoid " should be suspected of hog-cholera.